1-1-2005

Upright dieback disease of cranberry, Vaccinium macrocarpon Ait. : casual agents and infection courts.

Nora J. Catlin
University of Massachusetts Amherst

UPRIGHT DIEBACK DISEASE OF CRANBERRY, *VACCINIUM MACROCARPON* AIT.: CAUSAL AGENTS AND INFECTION COURTS

A Dissertation Presented

by

NORA J. CATLIN

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2005

Plant, Soil, and Insect Sciences

UPRIGHT DIEBACK DISEASE OF CRANBERRY, *VACCINIUM MACROCARPON* AIT.: CAUSAL AGENTS AND INFECTION COURTS

A Dissertation Presented

by

NORA J. CATLIN

Approved as to style and content by:

Frank L. Caruso, Chair

Wesley R. Autio, Member

Daniel R. Cooley, Member

Duane W. Greene, Member

Robert L. Wick, Member

Petrus L. Veneman, Department Head
Plant, Soil, and Insect Sciences

DEDICATION

To all who offered love, laughter, support, and patience throughout the duration of this graduate program: my amazing parents, family, and friends, including, of course, Jeffrey Chapman. My heartfelt thanks to you all.

ACKNOWLEDGMENTS

I would like to acknowledge the numerous individuals who provided assistance and materials which made many aspects of this research possible. Lisa A. Castlebury, David F. Farr, and Amy Y. Rossman (USDA ARS Systematic Botany and Mycology Laboratory, Beltsville, MD) provided isolates of *Phomopsis* as well as helpful suggestions. Dale Calaham (UMass Central Microscopy Facility), with funding from a grant from the National Science Foundation (NSF BBS 8714235), fixed and sectioned cranberry tissue for microscopical examination and allowed my use of the facilities and equipment. Rod Serres (Ocean Spray Cranberries, Inc.) generously provided assistance, plant tissue cultures, and the use of their laboratories and equipment. Mike DeGrandchamp, (DeGrandchamps Farms, South Haven, MI) donated blueberry plants for use in inoculation experiments. Cooperating growers A.D. Makepeace Cranberry Company, Card Cranberry Company, and Edgewood Cranberry Trust permitted me to make numerous trips to and collect many samples from their cranberry beds. Additionally, I would like to recognize the Cape Cod Cranberry Grower's Association and the USDA/CSREES Special Grant for financial support of this project.

Many thanks to my committee members, Frank L. Caruso, Wesley R. Autio, Daniel R. Cooley, Duane W. Greene, and Robert L. Wick, for their time, advice, guidance throughout these projects and the duration of my doctoral program. Special thanks to Frank L. Caruso for assistance with the identification of fungi.

Thanks to all at the UMass Cranberry Station for their help, suggestions, friendship over the course of these projects. Numerous seasonal staff assisted in many,

often tedious, aspects of these projects. Many thanks to Shelaugh Booth, Matt Clark, Robin Brese, Nick Caruso, Anissa Demers, Colleen Dugan, Katie Finely, Katie Hutchinson, Dave Janas, Liz Wade, and Kathy Wilson. Special thanks to Hilary Sandler for her time spent reading rough drafts and providing me with valuable editorial comments. Many happy thanks to Jane Mika for making our lab a more enjoyable place to be and for all of her assistance with my various projects.

ABSTRACT

UPRIGHT DIEBACK DISEASE OF CRANBERRY, *VACCINIUM MACROCARPON* AIT.: CAUSAL AGENTS AND INFECTION COURTS

SEPTEMBER 2005

NORA J. CATLIN, B.S., THE PENNSYLVANIA STATE UNIVERSITY

M.S., THE PENNSYLVANIA STATE UNIVERSITY

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Frank L. Caruso

The objective of this study was to determine the role of *Phomopsis vaccinii* in upright dieback disease of cranberry (*Vaccinium macrocarpon*). Specifically, the goals were to complete Koch's Postulates for *P. vaccinii* as a pathogen of cranberry, determine infection courts, evaluate the pathogenicity of various isolates of *P. vaccinii* and isolates of non-*P. vaccinii Phomopsis* from cranberry and blueberry (*Vaccinium corymbosum*), and determine which cranberry tissues *P. vaccinii* infects and colonizes.

Koch's Postulates were completed using tissue-cultured and rooted cuttings of two cultivars. It was therefore concluded that *P. vaccinii* is a causal agent of upright dieback disease.

Various infection courts were tested by conducting inoculations of different tissue using different wounding techniques. It was determined that, while non-wounded

plants occasionally developed symptoms, stem-pierce wounds resulted in infection of more plants and typically greater tissue death than other wound techniques. A higher percent of plants on which current-year growth was inoculated developed symptoms compared to plants on which 1-yr-old growth was inoculated. It was concluded that current-year growth in spring was the most susceptible growth stage, although plants can be infected throughout the season if wounded. It was observed that only current-year growth was affected when infection occurred in the current-year growth, and infection did not progress to adjacent runners or uprights if the infection occurred in the 1-yr-old growth.

It was determined that isolates of *P. vaccinii* and non-*P. vaccinii* isolates of *Phomopsis* could result in symptom development on tissue-cultured cranberry plants and rooted cuttings of cranberry. More *P. vaccinii* isolates resulted in disease development than other *Phomopsis* sp. isolates. A few isolates did not result in symptom development on any inoculated plant, or resulted in symptom development on only a low percent of plants. Since these isolates were regularly isolated from symptomless tissue, it is probable that these isolates are non-parasitic endophytes of cranberry plants.

P. vaccinii-inoculated tissue-cultured plants were examined microscopically, and *P. vaccinii* was observed throughout necrotic leaf tissue and in vascular stem tissue. These observations indicate that *P. vaccinii* is a vascular pathogen. It is expected that the fungus infects succulent growth and progresses from leaf tissue into the stem tissue, or infects through stem wounds, eventually colonizing vascular tissue.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

General Introduction

The American cranberry, *Vaccinium macrocarpon* Ait., is one of a few native crops to North America (Eck 1990). The cranberry is an evergreen, non-deciduous, trailing vine with lateral runners reaching lengths of 1-2 m and uprights, or vertical branches. reaching 5-15 cm that are either vegetative or fruit bearing. The upright is terminated by a mixed bud that produces new shoot growth and the flowers, which are borne on the new shoot growth, in the spring. Cranberry vines form a dense mat on the surface of the bed, and, for commercial production, are grown in moist, well-drained, acidic soil in peat-bog beds, or in some cases sandy-soil beds (DeMoranville and Sandler 2000), which are surrounded by ditches and dikes and located near a substantial water source. The cranberry is a long-lived plant, and well-managed beds can remain productive for a considerable length of time; in Massachusetts and New Jersey, some beds have been in production for over 100 years (Oudemans et al. 1998).

In the United States, cranberries are predominantly produced in five states: Wisconsin, Massachusetts, Oregon, New Jersey, and Washington (New England Agricultural Statistics Service 2005). In 2004, the cranberry crop in the United States totaled 290,300,000 kg, harvested from a total of 15,864 ha. Of the national cranberry crop, Wisconsin produced 55%, Massachusetts produced 28%, Oregon produced 8%, and New Jersey produced 6%. In Massachusetts, approximately 81,650,000 kg were

harvested from 5,706 ha with a value of approximately $63 million, or approximately $11,040 per ha.

Numerous factors can affect the yield of cranberry beds including weather, the availability of water and nutrients, diseases, insects, and weeds (Mahr and Moffitt 1994, Caruso and Ramsdell 1995, DeMoranville et al. 1997). Fruit rot, caused by a complex of fungi, is consistently the most troublesome disease problem for growers and can result in substantial crop loss (Oudemans et al. 1998). Other vine and foliar diseases, such as Phytophthora root rot, fairy ring disease, and upright dieback disease, can also periodically cause considerable crop loss in Massachusetts (Mahr and Moffitt 1994, Caruso and Ramsdell 1995). Due to the high value of the cranberry crop, it is critically important to reduce losses by understanding the biology of causal agents as well as the epidemiology of diseases and determining optimal disease management practices.

One vine disease of cranberry of which little is known is upright dieback disease, first reported in 1966 (Friend and Boone 1968). Upright dieback has been reported to occur in all areas of cranberry cultivation and can be a persistent problem on affected cranberry beds (Caruso and Ramsdell 1995). The typical symptom of upright dieback is tip dieback of the cranberry upright. Leaves at the tip of an affected upright first become discolored, taking on a yellowish cast, then turn orange to brown as the tissue becomes necrotic. The dieback symptom progress down the stem, but are rarely observed to affect runner tissue. Roots of affected plants remain healthy. Leaves typically are retained during the season of infection, and diseased uprights appear dispersed among healthy uprights in an affected cranberry bed. Little is known about

the relative resistance or susceptibility of various cultivars to the disease, though most cultivars appear susceptible (Caruso and Ramsdell 1995).

Several fungi have been routinely cultured from affected uprights, including *Aureobasidium pullulans* (de Bary) G. Arnaud, *Colletotrichum acutatum* J.H. Simmonds, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., *Fusicoccum putrefaciens* Shear, *Gloeosporium minus* Shear, *Pestalotia vaccinii* (Shear) Guba, and *Phomopsis vaccinii* Shear, but the primary causal agent remained unproven (Friend and Boone 1968, Caruso unpublished data). Many of these fungi are also known causal agents of fruit rot – *C. acutatum*, *C. gloeosporioides*, *F. putrefaciens*, and *Phomopsis vaccinii* are all associated with field and/or storage rots of cranberry (Oudemans et al. 1998).

Koch's Postulates to prove pathogenicity have been completed with *Synchronoblastia crypta* Uecker & Caruso (Uecker and Caruso 1988). However, this fungus has rarely been isolated from uprights in Massachusetts over the past ten years, and it currently is not believed to be a significant factor in the occurrence of upright dieback (Caruso, unpublished data). *Phomopsis vaccinii* has long been assumed to be a causal agent due to its frequent recovery from diseased uprights (Friend and Boone 1968, Caruso and Ramsdell 1995). Friend and Boone (1968) isolated *P. vaccinii* predominantly from diseased stems, but also isolated *F. putrefaciens* and species of *Alternaria*, *Epicoccum*, and *Fusarium*. In their studies, only *P. vaccinii* and *Fusarium* spp. were isolated more frequently from diseased stems than apparently healthy stems. Since *Fusarium* spp. were only isolated occasionally from diseased and healthy stems, it was concluded that *P. vaccinii* was most likely the pathogen. Another reason why *P.*

vaccinii has been assumed to be the causal agent of upright dieback is due to its documented role in twig blight and canker diseases of highbush blueberry, *Vaccinium corymbosum* L. (Wilcox 1939, Weingartner and Klos 1975, and Parker and Ramsdell 1977).

Research Objectives

The main focus objective of the research presented in this dissertation was to determine the role of *Phomopsis vaccinii* in upright dieback disease of cranberry. Specific objectives included:

i. determine pathogenicity of *P. vaccinii*,

ii. determine the infection courts of the fungus,

iii. determine the pathogenicity of various isolates of *P. vaccinii* and *Phomopsis* sp. isolated from cranberry, *V. macrocarpon*, and blueberry, *V. corymbosum*, and

iv. determine which types of cranberry tissues *P. vaccinii* infects and colonizes.

CHAPTER 2

PROOF OF PATHOGENICITY OF *PHOMOPSIS VACCINII*

Introduction

Although numerous fungi are commonly isolated from cranberry, *Vaccinium macrocarpon*, stems showing symptoms of upright dieback, *Phomopsis vaccinii* has been assumed to be the causal agent of the disease. This assumption is based on the frequent isolation from diseased stems and its known association with twig dieback and canker diseases of highbush blueberry, *Vaccinium corymbosum* (Friend and Boone 1968, Wilcox 1939, Weingartner and Klos 1975, and Parker and Ramsdell 1977). Despite the information pointing to *P. vaccinii* as the causal agent of upright dieback, Koch Postulates have not been completed. Koch's Postulates, or Koch's Rules, stipulate a series of steps which must be completed to verify whether a suspected pathogen is the causal agent of the disease of interest (Agrios 1988). The steps of Koch's Postulates are: (i) find the pathogen associated with diseased plants, (ii) isolate the pathogen from diseased tissue and obtain a pure culture, (iii) inoculate the pathogen into healthy plants and observe the same symptoms on the inoculated plant as observed in the field, and (iv) re-isolate the pathogen from the inoculated plant and ensure that the culture has the same characteristics as the culture from which the inoculum was produced.

In preliminary studies, various phenological stages of the host were inoculated with *P. vaccinii* using several techniques without success (Catlin and Caruso, unpublished data). The objective of this portion of the study was to determine causal

agents of upright dieback by inoculating tissue-cultured cranberry plants and rooted cuttings of cranberry plants. To eliminate problems associated with infested plant stock, possible latent infections, cross contamination, and genetic variability, tissue-cultured plants were first used for inoculation trials. Inoculations of tissue-cultured cranberry plants were followed by inoculations of rooted cuttings of cranberry plants in the greenhouse.

Materials and Methods

Tissue-cultured Cranberry Plants

Inoculations of tissue-cultured plants were conducted with cv. Stevens and repeated with cv. Early Black. Both plant tissue cultures were originally isolated from plants in the variety collection of Washington State University Long Beach Research and Extension Unit, which originated from fingerprinted stock from the Blueberry and Cranberry Research Center, Chatsworth, NJ.

Fresh transfers of cranberry plant cultures were grown on 60 ml of woody plant media (WPM) (Lloyd and McCown 1980) in 7.62 x 7.62 x 10.16 cm Magenta GA7 containers and caps for 3 to 4 weeks. From these containers, individual cuttings were transferred to 10 ml WPM in 22 x 92 mm Kimble shell vials with KimKap™ polypropylene closures. Transfers were maintained in the shell vials for 10 to 20 days before inoculation. All plants were maintained under 40W cool white fluorescent lights in a room with an average temperature of 25.3˚C and relative humidity of 45.9%.

Agar plugs of mycelia, 3 mm diameter, taken from the growing edges of 3 to 5 day old fungal colonies maintained on half-strength potato dextrose agar (½PDA) were

used as inoculum. Agar plugs of sterile ½ PDA were used in controls. The two fungi that were used for the inoculations are listed in Table 2.1. Three inoculation methods were used: (i) plants were not wounded and the inoculum was placed on a leaf towards the top of the plant, (ii) plants were wounded by cutting and removing the upper portion of the stem and the inoculum was placed on an unwounded leaf toward the top of the plant, or (iii) plants were wounded by piercing a leaf twice with a sterile needle (approximately 0.5 mm diameter) and the inoculum was placed on the wounded leaf.

Each treatment was replicated 8 times using each fungal isolate and the sterile agar control. A single inoculated plant was considered a replicate. Plants were arranged in a completely random design. Observations were recorded approximately every 7 days after inoculation for at least 50 days. At the termination of the experiments, a small portion of diseased stem tissue and, in a few cases, symptomless stem tissue was selected randomly. The stem segments were surface disinfested by soaking for 15 s in 70% ethanol (ETOH), plated on half-strength acidified potato dextrose agar (½ APDA), and observed for fungal growth after 1 to 2 weeks of incubation at room temperature. Additionally, dry shoot weight was recorded. The data from each trial were subjected to analysis of variance (ANOVA), and the treatment means were separated using Kramer-adjusted Tukey's honestly significant difference (HSD) test (SAS V 9.1, SAS Institute). To better meet ANOVA model assumptions, data from the 'Stevens' trial were transformed prior to analysis using a square root transformation.

Cranberry Rooted Cuttings

Inoculations were conducted with rooted cuttings of 'Early Black' and 'Stevens' plants maintained in greenhouse conditions. Agar plugs consisting of mycelia (3 mm diameter) were taken from the growing edges of 3 to 5 day old fungal colonies maintained on ½ PDA and used as inoculum, and agar plugs of sterile ½ PDA were used in controls. The same fungal isolates that were used for the tissue culture inoculations were used in the greenhouse trials (Table 2.1).

The inoculation procedure used was similar to the procedure described by Weingartner and Klos (1975) for inoculation of highbush blueberry with *P. vaccinii*. Young tissue approximately 3 to 4 cm from the tip of an upright was wounded. Wounds were produced by removing 3 to 4 leaves and piercing the stem once with a sterile needle (approximately 0.5 mm diameter). The agar plug was placed over the wound, wrapped in sterile Kimwipes® (approximately 2x8 cm) moistened with sterile distilled water, and sealed with Parafilm®. Each treatment was replicated 10 times for each cultivar; a single inoculated plant was considered one replicate. The inoculated plants were arranged on a greenhouse bench in a completely random design.

Observations were recorded every 7 to 14 days after inoculation for approximately 3 months for both the 'Early Black' and the 'Stevens' trials. The 'Stevens' trial was initiated in August 2002 and the 'Early Black' trial was initiated in October 2002. After observations, plants were overwintered by placing in a cooler and re-inspected in the summer of 2003 for symptoms after the plants had completed their seasonal growth.

At the termination of the experiments, approximately 12 months after inoculation in the 'Stevens' trial and approximately 10 months after inoculation in the 'Early Black' trial, diseased tissue was sampled and surface sterilized by soaking for 10 s in 70% ETOH followed by 2 min in 0.5% sodium hypochlorite (NaOCl) plus Tween80® solution. After surface disinfestation, the samples were plated onto ½ APDA. Additionally, plants were harvested and fresh weights were recorded. The fresh weight data were subjected to analysis of variance (ANOVA), and the treatment means were separated using Kramer-adjusted Tukey's HSD test (SAS V 9.1, SAS Institute).

Results

Tissue-cultured Cranberry Plants

Symptoms

All 'Stevens' tissue-cultured plants inoculated with *P. vaccinii* showed dieback symptoms – discoloration of the stem and leaves beginning at the point of inoculation, then progressing outward eventually constricting and killing the tissue (Figure 2.1). Examples of 'Stevens' tissue-cultured plants exhibiting symptoms after inoculation with *P. vaccinii* are shown in Figures 2.2 to 2.4. Symptoms were first observed 12.5 days ± 3.6 after inoculation on the plants inoculated with the stem-cut procedure, 19.0 days ± 3.8 on the non-wounded plants, and 22.1 days ± 5.1 on the plants inoculated with the leaf-pierce procedure. New buds and shoots often developed on inoculated plants, and, in most cases, this new growth also became infected and killed by *P. vaccinii.* By 50 days after inoculation, 88% of the 'Stevens' plants inoculated with the leaf pierce and

no-wound methods, and 100% of the plants inoculated with the stem-cut method were dead.

All 'Early Black' tissue-cultured plants inoculated with *P. vaccinii*, except one non-wounded plant, showed dieback symptoms (Figure 2.5). Examples of 'Early Black' tissue-cultured plants showing symptoms after inoculation with *P. vaccinii* are shown in Figures 2.6 to 2.8. Plants inoculated with the leaf-pierce procedure started exhibiting symptoms 5.0 days ± 0.0 after inoculation, while plants inoculated with the stem-cut procedure began showing symptoms 14.1 days ± 2.5 after inoculation, and non-wounded plants started showing symptoms 31.9 days ± 5.5 after inoculation. Like *P. vaccinii*-inoculated 'Stevens' plants, *P. vaccinii*-inoculated 'Early Black' plants often developed new buds and shoots which, in most cases, also became infected and killed by *P. vaccinii*. By 50 days after inoculation, 100% of the 'Early Black' plants inoculated with the leaf-pierce and the stem-cut method, and 38% of the plants inoculated with the no-wound method were dead.

Of all the 'Stevens' plants inoculated with *Discula* sp., 25.0% showed dieback symptoms (Figure 2.1). Two plants inoculated with the stem-cut procedure first exhibited symptoms 9.0 days ± 3.0 after inoculation, and one non-wounded plant first exhibited symptoms 36 days after inoculation. Of the *Discula* sp.-inoculated 'Early Black' plants, one stem-pierce plant and one stem-cut plant (8.3%) showed dieback symptoms starting 42 days after inoculation (Figure 2.5). Other than one 'Stevens' stem-cut plant, no plants of either cultivar were dead by 50 days after inoculation with *Discula* sp. For both cultivars, new buds and shoots were often produced on the *Discula* sp.-inoculated plants, particularly on wounded plants. Another response to

inoculation with *Discula* sp. was observed: in numerous replicates, the leaf on which the inoculum was placed became necrotic and dropped off.

Tissue reddening was observed around the leaf wounds on inoculated control plants of both cultivars. Control plants also responded to inoculation by producing new buds and shoots on wounded 'Stevens' plants and on most 'Early Black' plants. No dieback symptoms were observed on any of the 'Stevens' control plants, and one leaf-pierced 'Early Black' control plant showed a dieback-like symptom 34 days after inoculation (Figures 2.1 and 2.5). The one control plant that exhibited symptoms was not dead by 50 days after inoculation. Examples of healthy 'Stevens' and 'Early Black' tissue-cultured plants inoculated with the sterile agar control are shown in Figures 2.9 and 2.10, respectively.

Recovery of fungi

P. vaccinii was recovered from the plated samples of diseased plant tissue of both cultivars. *Discula* sp. was recovered from the diseased plant tissue as well as from symptomless tissue of both cultivars. No fungi were recovered from the one 'Early Black' control plant that exhibited dieback symptoms, and no fungi were recovered from any of the healthy control plants.

Dry weight

Mean dry shoot weight of 'Stevens' plants inoculated with either *P. vaccinii*, *Discula* sp. or a sterile agar control was significantly different ($p < 0.0001$). Since there was no significant interaction between the isolate and the wound method, the combined data of the wound methods for each isolate is presented. Dry weight of plants

inoculated with *P. vaccinii* was significantly different from the dry weight of the plants inoculated with *Discula* sp. and the control plants, and the dry weight of control plants and plants inoculated with *Discula* sp. was not significantly different. Dry weight for plants inoculated with *P. vaccinii* was 17.0 mg ± 1.2, dry weight for plants inoculated with *Discula* sp. was 25.9 mg ± 1.6, and dry shoot weight for control plants was 21.8 mg ± 1.3 (Figure 2.11).

Dry shoot weight of 'Early Black' plants inoculated with *P. vaccinii*, *Discula* sp. or a sterile agar control was also significantly different ($p < 0.0001$). The dry weight of *P. vaccinii*-inoculated 'Early Black' plants was significantly different from that of *Discula* sp.-inoculated plants and control plants, and dry weight of *Discula* sp-inoculated plants and control plants were not significantly different. Dry shoot weight of 'Early Black' plants inoculated with *P. vaccinii* was 21.7 mg ± 3.0, while *Discula* sp.-inoculated plants and control plants had dry weights of 50.8 mg ± 2.7 and 50.9 mg ± 2.5, respectively (Figure 2.12). There was a significant isolate by wound method interaction in the 'Early Black' trial ($p = 0.0019$). For the plants inoculated with *P. vaccinii,* the shoots inoculated with the leaf-pierce method had significantly less dry shoot weight than the plants inoculated with the stem-cut and no-wound procedures. The *P. vaccinii* -inoculated leaf-pierced plants weighed 7.8 mg ± 0.6, and the stem-cut plants and the non-wounded plants weighed 23.8 mg ± 5.1 and 33.6 mg ± 3.8, respectively.

Greenhouse Trials

Symptoms

Eighty percent of *P. vaccinii*-inoculated 'Stevens' rooted cuttings showed typical dieback symptoms – discoloration and death starting at the tip and progressing down the stem (Figure 2.13). Dieback symptoms were observed 7 days after inoculation, and by 14 days after inoculation, the current-year growth on most inoculated uprights was necrotic. Tissue death never progressed into the past-year's growth on affected uprights. Control plants and plants inoculated with *Discula* sp. showed no response to inoculation and remained symptomless. No changes in symptoms were observed in any of the plants after overwintering. An example of a *P. vaccinii*-inoculated 'Stevens' rooted cutting showing symptoms and a healthy 'Stevens' control plant are shown in Figures 2.14 and 2.15, respectively.

Seventy percent of inoculated 'Early Black' plants showed typical dieback symptoms (Figure 2.13). Leaf reddening was observed 7 to 10 days after inoculation, and tissue death was observed 22 days after inoculation. As with the inoculated 'Stevens' plants, there was no progression of diseased tissue past the current-year growth in the 'Early Black' plants. No symptoms were observed on control plants or plants inoculated with *Discula* sp. No changes in symptoms were observed in any of the plants after overwintering. An example of a *P. vaccinii*-inoculated 'Early Black' rooted cutting showing symptoms and a healthy 'Early Black' control plant are shown in Figures 2.16 and 2.17, respectively.

Recovery of Fungi

P. vaccinii was recovered from the sampled diseased tissue of both 'Stevens' and 'Early Black' rooted cuttings. Samples from symptomless plants were not selected for plating.

Fresh Weight

'Stevens' plants inoculated with *P. vaccinii* produced numerically the lowest fresh biomass, 1.78 g ± 0.20, followed by plants inoculated with *Discula* sp., 2.18 g ± 0.22, and control plants, 2.29 g ± 0.26 (Figure 2.18). However, these differences were not statistically significant. In the 'Early Black' trial, the average fresh weight of the *P. vaccinii*-inoculated plants, 1.76 g ± 0.20, was significantly less than that of the control plants, 2.98 g ± 0.25 ($p = 0.0141$) (Figure 2.19). Fresh biomass of plants inoculated with *Discula* sp., 2.57 g ± 0.36, was not significantly different from the fresh biomass of either the *P. vaccinii*-inoculated plants or the control plants.

Discussion

The dieback symptoms observed on the individual tissue culture plants and the rooted cuttings in the greenhouse were similar to the symptoms observed in the field. *P. vaccinii* inoculation of tissue-cultured plants produced dieback symptoms on all 'Stevens' plants and all but one 'Early Black' plant. Inoculation of rooted cuttings with *P. vaccinii* produced dieback symptoms on 80% of 'Stevens' plants and 70% of 'Early Black' plants. All controls in the two rooted-cutting trials remained symptomless, and

all controls, with the exception of one 'Early Black' tissue-cultured plant, remained symptomless in the tissue culture trials.

P. vaccinii was recovered from diseased tissue sampled from the *P. vaccinii*-inoculated tissue-cultured plants and from the diseased tissue sampled from the *P. vaccinii*-inoculated rooted cuttings. No fungi were recovered from the symptomless tissue-cultured control plants or the one tissue-cultured 'Early Black' control plant that exhibited dieback symptoms. It is unknown why a dieback-like symptom occurred in this control replicate. No contamination, fungal or bacterial, was present. Dieback symptoms could be expressed if an unhealthy tissue-cultured plant was selected for inoculation or if the plant growth medium became dry. These experiments demonstrated that *P. vaccinii* is a pathogen of cranberry stems. This is the first reported successful completion of Koch's Postulates for *P. vaccinii* as a causal agent of upright dieback disease of cranberry.

Inoculation with *Discula* sp. produced symptoms in only one stem-pierce and one stem-cut tissue-cultured plant in the 'Early Black' trial, and two stem-cut tissue-cultured plants and one non-wounded plant showed dieback symptoms in the 'Stevens' trial. Tissue-cultured plants have little to no cuticle and the environment in the tissue-culture vial is highly favorable for fungal growth. For these reasons, tissue culture plants provide an extreme in host sensitivity when used for pathogenicity testing. Therefore, it should be considered that *Discula* sp. would not, or would only rarely, cause dieback symptoms under field conditions. This conclusion is supported by the result that no *Discula* sp.-inoculated rooted cuttings exhibited dieback symptoms in either the trial.

Discula sp. was recovered from diseased and healthy tissue sampled from the inoculated tissue-cultured plants. It is interesting to note that *Discula* sp. was recovered from surface disinfested symptomless tissue samples of tissue-cultured plants. The fungal recovery from symptomless tissue-cultured plant tissue along with the low incidence of disease in the *Discula* sp.-inoculated tissue-cultured plants and rooted cuttings suggests the possibility that this *Discula* sp. isolate, 93009C, is generally a non-pathogenic endophyte that occasionally, under extreme conditions, can cause dieback symptoms. Species of *Discula* have been reported as endophytes for other woody plants, including Japanese beech, *Fagus crenata*, European beech, *Fagus sylvatica*, and oaks, *Quercus cerris* and *Quercus garryana* (Sahashi et al. 1999, Sahashi et al. 2000, Toti et al. 1993, Ragazzi et al. 1999 and Wilson and Carroll 1994). However, since only a small number of symptomless tissue-cultured plant samples were selected for plating and symptomless tissue of rooted cuttings were not selected for plating, more information is needed to confirm the suggestion that *Discula* sp. is an endophyte of cranberry.

In the tissue-culture trials, wounded plants inoculated with *P. vaccinii* were generally affected before non-wounded plants, although non-wounded plants eventually developed symptoms in most cases. In both the 'Early Black' and 'Stevens' tissue-cultured plant trials, only four plants were affected by *Discula* sp. inoculation, and all but one of those affected plants were wound-inoculated plants. It can therefore be concluded that wound-inoculation techniques are the most efficient procedure to use for inoculations of cranberry tissue culture plants. Of the two different inoculation methods

used in the tissue-culture trials, the leaf-pierce method and the stem-cut method, the stem-cut method was the easiest to execute using sterile technique.

In the field, it is observed that 'Early Black' plants appear to be more susceptible to upright dieback than 'Stevens' plants (Caruso, personal communication). In the tissue-culture trials, *P. vaccinii* infection occurred more rapidly after inoculation in the 'Early Black' trial than the 'Stevens' trial, particularly in the wounded plants. In general, the majority of plants were dead by 50 days after inoculation with the two wound-inoculation methods for both trials. However, in the 'Stevens' trial, more non-wounded plants were dead by 50 days after inoculation (87.5%) than in the 'Early Black' trial (37.5%). Symptoms were first observed in the inoculated 'Stevens' and 'Early Black' rooted cuttings at approximately the same length of time after inoculation. However, dissimilar to the tissue-culture trials, tissue death of *P. vaccinii* -inoculated 'Early Black' plants was observed later than *P. vaccinii* -inoculated 'Stevens' plants. Symptoms of tissue death were observed 22 days after inoculation for *P. vaccinii* -inoculated 'Early Black' plants, while symptoms of tissue death were observed 7 days after inoculation for *P. vaccinii*-inoculated 'Stevens' plants. Since the trials were not conducted concurrently, it is difficult to draw a strong conclusion on the relative susceptibility of the two cultivars. The 'Stevens' tissue-culture trial was initiated in March 2002 and the 'Early Black' trial was initiated in June 2002. For the rooted-cutting trials, the 'Stevens' trial was initiated in August 2002 and the 'Early Black' trial was initiated in October 2002. While the 'Early Black' rooted cuttings were maintained under lights (12 h of light per day), these plants were not growing as vigorously as the

plants in the 'Stevens' trial, and this difference in growth stage may have affected the symptom development in the different trials.

Inoculated tissue-cultured plants continue to grow in height and often produce shoots until infected. Similarly, uprights of rooted cuttings exhibiting dieback ceased to grow while non-inoculated upright or non-infected upright continued to grow. For these reasons, dry shoot weight of inoculated tissue-cultured plants and fresh shoot weight of rooted cuttings were taken as a measure of disease severity for each treatment. For both tissue-culture trials, plants inoculated with *P. vaccinii* weighed significantly less than plants inoculated with *Discula* sp. and the control plants – a result of the high number of affected replicate plants and extensive tissue death caused by *P. vaccinii*. Fresh weight was lower for *P. vaccinii*-inoculated plants than control plants or plants inoculated with *Discula* sp. in both the 'Early Black' and 'Stevens' trials. However, only in the 'Early Black' trial was the fresh weight of the *P. vaccinii*-inoculated plants significantly different from the control treatment. The 'Early Black' trial was initiated in October of 2002, while the 'Stevens' trial was initiated in August 2002. Dieback symptoms did not progress past the current-year tissue, thus the tissue damage was minimal and the other uprights and runners continued growth. Since both experiments were terminated at the same time, the 'Stevens' plants had a longer amount of time for growth, thus reducing the differences between the weight of plants exhibiting symptoms and symptomless plants.

As mentioned above, tissue-cultured plants offer an extreme in host sensitivity. While the inoculations of the rooted cuttings were successful, tissue-cultured plants offer a clean, convenient, quick, and space-efficient method for testing pathogenicity.

When both the tissue culture and the rooted cutting trials were considered, it was concluded that *Discula* sp. was not a pathogen of cranberry. However, the results of the *Discula* sp. tissue culture inoculations helped implicate *P. vaccinii* as a pathogen when only the tissue culture procedure was considered. The results of the *Discula* sp. inoculations provided the information that the sensitivity of the tissue-cultured cranberry plants was not such that the plants would be negatively affected by the addition of any microorganism. Thus, based on these results, tissue-cultured plants are a valid and efficient method available for pathogenicity tests. Numerous researchers have reported the use of tissue-cultured plants for *in vitro* studies of pathogenicity or host resistance. Examples include the development of *in vitro* systems to study host-pathogen interactions (Zilkah et al. 1999, Abdollahi at al. 2004, Santos et al. 2005), the detection of pathogenic strains (Scheck et al. 1997), determination of resistance responses and resistance screening (Spanos et al. 1997, Hammerschlag 1988).

Table 2.1. Fungi used in proof-of-pathogenicity trials.

Isolate	Fungus	Host	Tissue	Origin
98023	*Phomopsis vaccinii*	*Vaccinium macrocarpon*	Stem	MA
93009C	*Discula* sp.[a]	*Vaccinium macrocarpon*	Stem	MA

[a] This isolate was originally identified as *Phomopsis* sp., but was recently determined to be morphologically similar to species of *Discula*, though not similar based on sequence analysis of the large subunit of ribosomal DNA (Castlebury, personal communication). Until a more suitable name is defined and for the purpose of this dissertation, this isolate will be referred to as *Discula* sp.

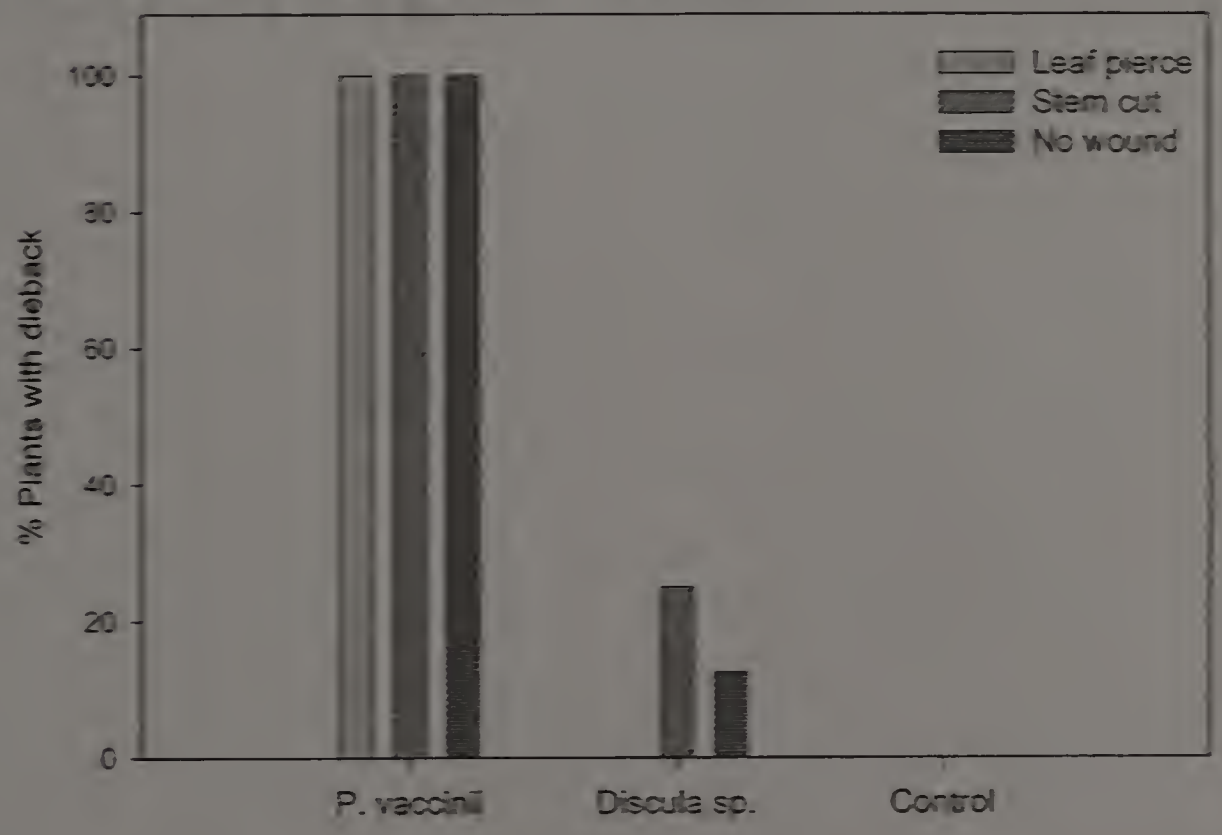


Figure 2.1. Percent of 'Stevens' plants showing upright dieback symptoms after inoculation with *P. vaccinii*, *Discula* sp., or a sterile agar control using three wound-inoculation techniques (N=8).

Figure 2.2. Tissue-cultured 'Stevens' plant exhibiting symptoms 16 days after inoculation with *P. vaccinii* using a no-wound inoculation procedure.

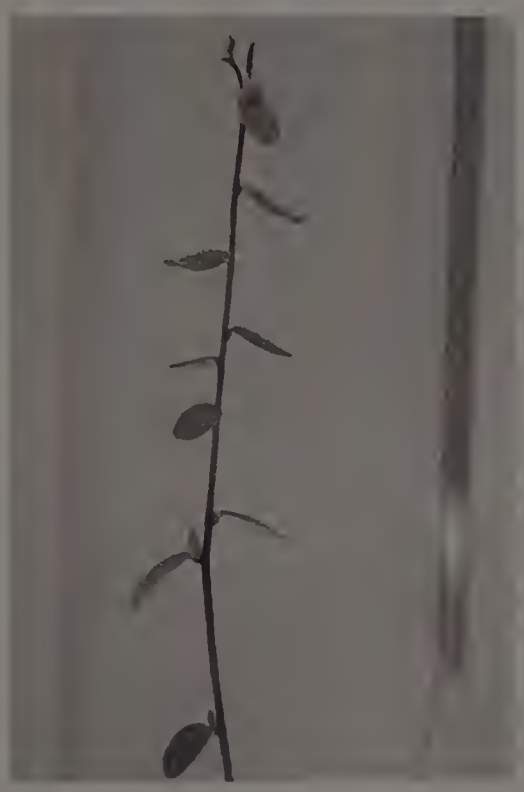


Figure 2.3. Tissue-cultured 'Stevens' plant exhibiting symptoms 22 days after inoculation with *P. vaccinii* using a stem-cut inoculation procedure.

Figure 2.4. Tissue-cultured 'Stevens' plant exhibiting symptoms 22 days after inoculation with *P. vaccinii* using a stem-pierce inoculation procedure.

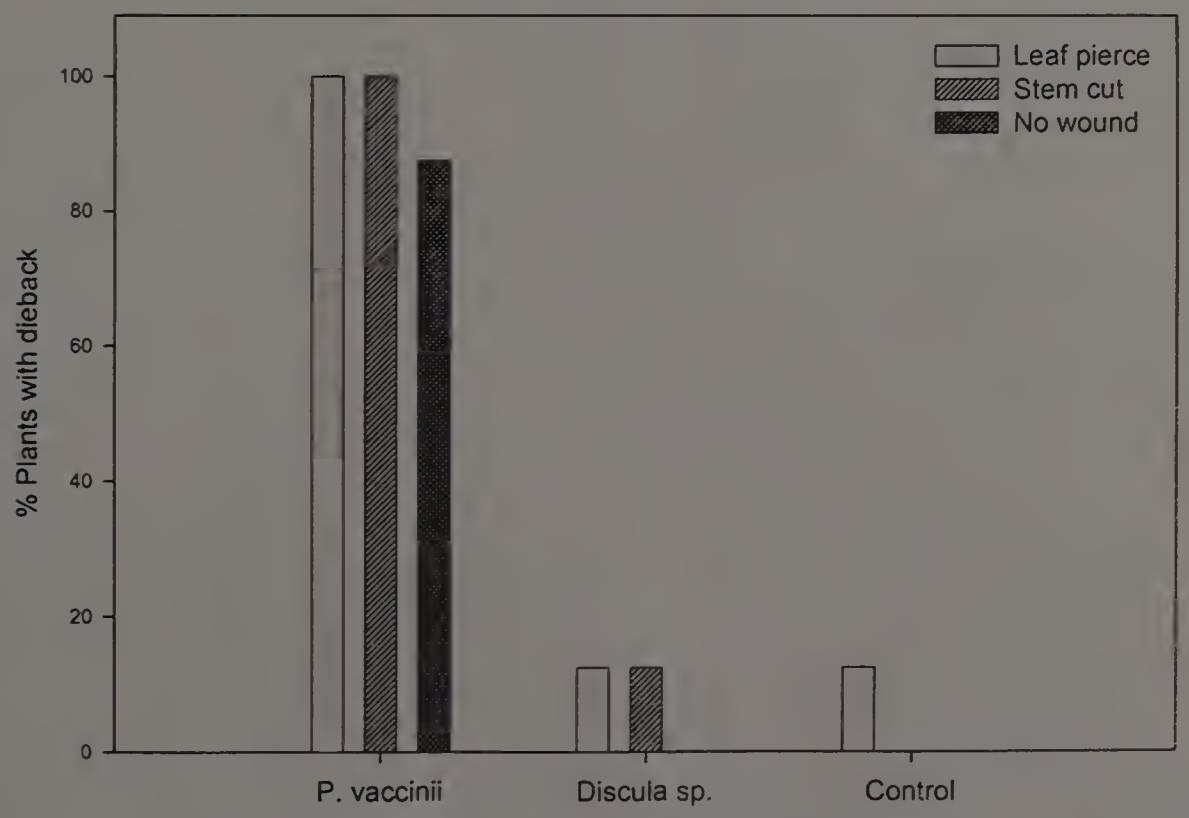
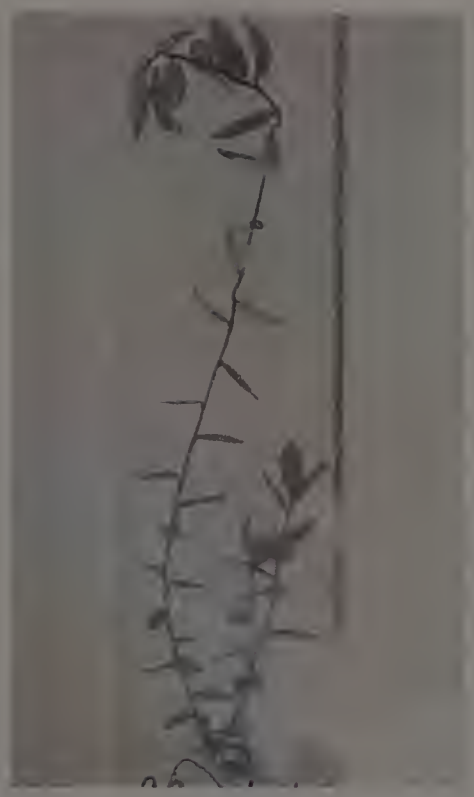


Figure 2.5. Percent of 'Early Black' plants showing upright dieback symptoms after inoculation with *P. vaccinii*, *Discula* sp., or a sterile agar control using three wound-inoculation techniques (N=8).



Figures 2.6. Tissue-cultured 'Early Black' plant exhibiting symptoms 50 days after inoculation with *P. vaccinii* using a no-wound inoculation procedure.

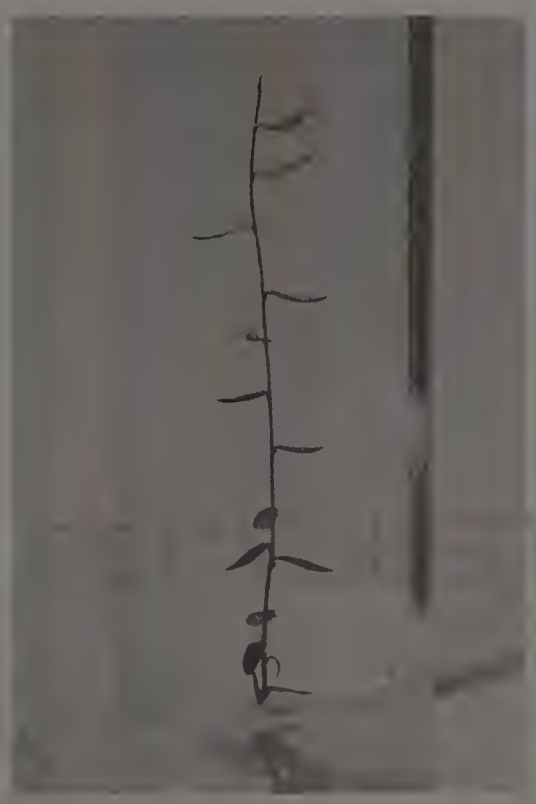


Figure 2.7. Tissue-cultured 'Early Black' plant exhibiting symptoms 14 days after inoculation with *P. vaccinii* using a stem-cut inoculation procedure.

Figure 2.8. Tissue-cultured 'Early Black' plant exhibiting symptoms 9 days (left), 14 days (center), and 27 days (right) after inoculation with *P. vaccinii* using a leaf-pierce inoculation procedure.

Figure 2.9. Tissue-cultured 'Stevens' plant 22 days after inoculation with a sterile agar control using a stem-cut inoculation procedure.

Figure 2.10. Tissue-cultured 'Early Black' plant 50 days after inoculation with a sterile agar control using a no-wound inoculation procedure.

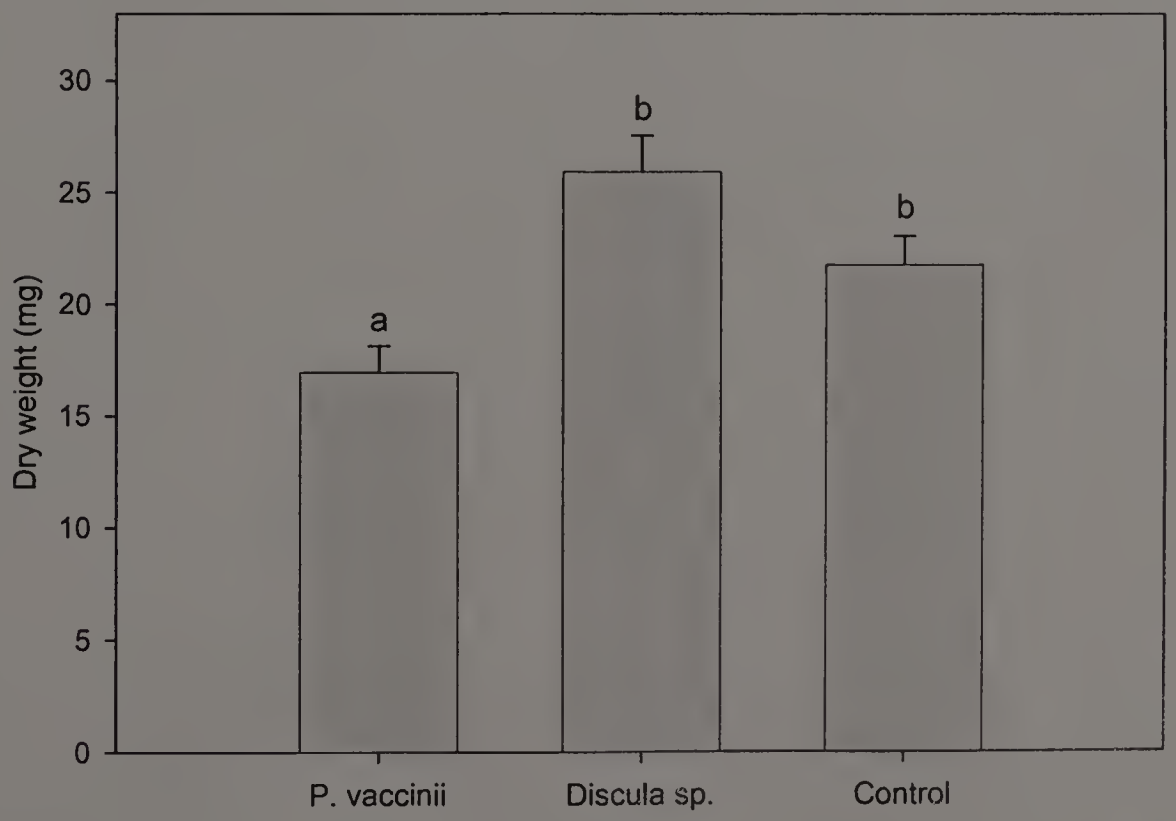


Figure 2.11. Mean dry shoot weight of 'Stevens' tissue culture plants inoculated with *P. vaccinii*, *Discula* sp., or a sterile agar control (N=24). Vertical bars represent standard error of means. Means with similar letters are not significantly different according to Kramer-adjusted Tukey's HSD (p = 0.05).

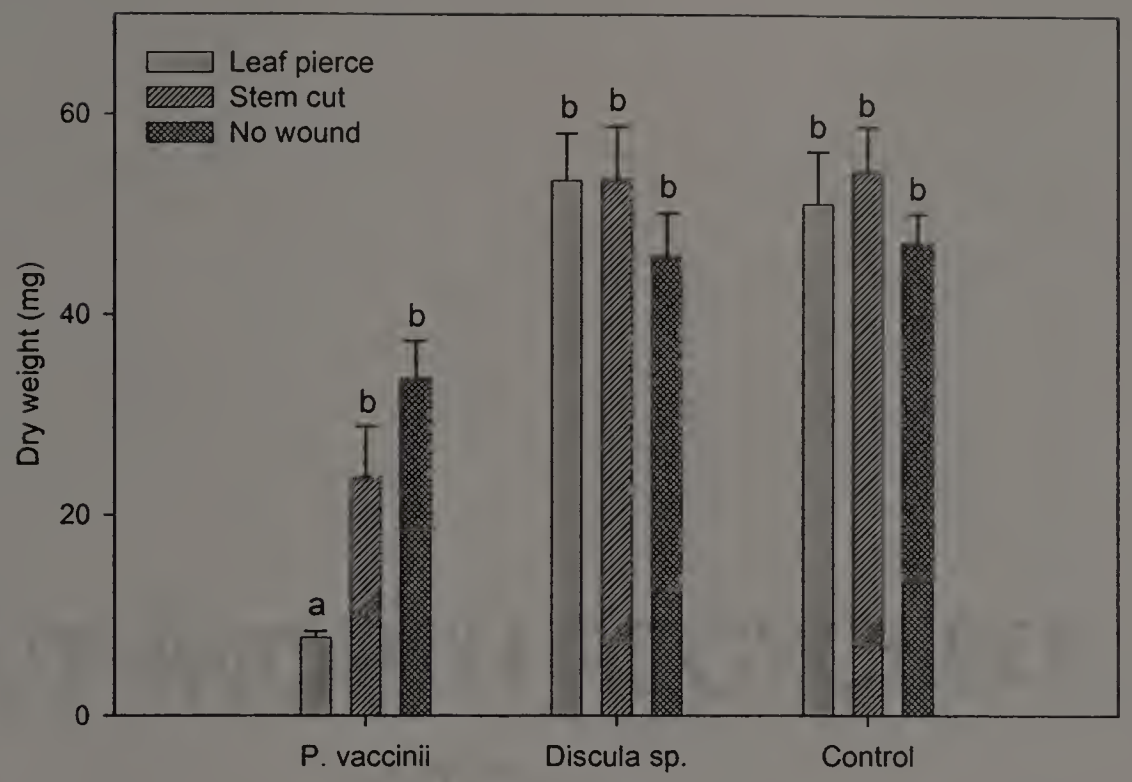


Figure 2.12. Dry shoot weight of 'Early Black' tissue-cultured plants inoculated with *P. vaccinii*, *Discula* sp., or a sterile agar control using different wound-inoculation techniques (N=8). Vertical bars represent standard error of means. Within each isolate, means with similar letters are not significantly different according to Kramer-adjusted Tukey's HSD (p = 0.05).

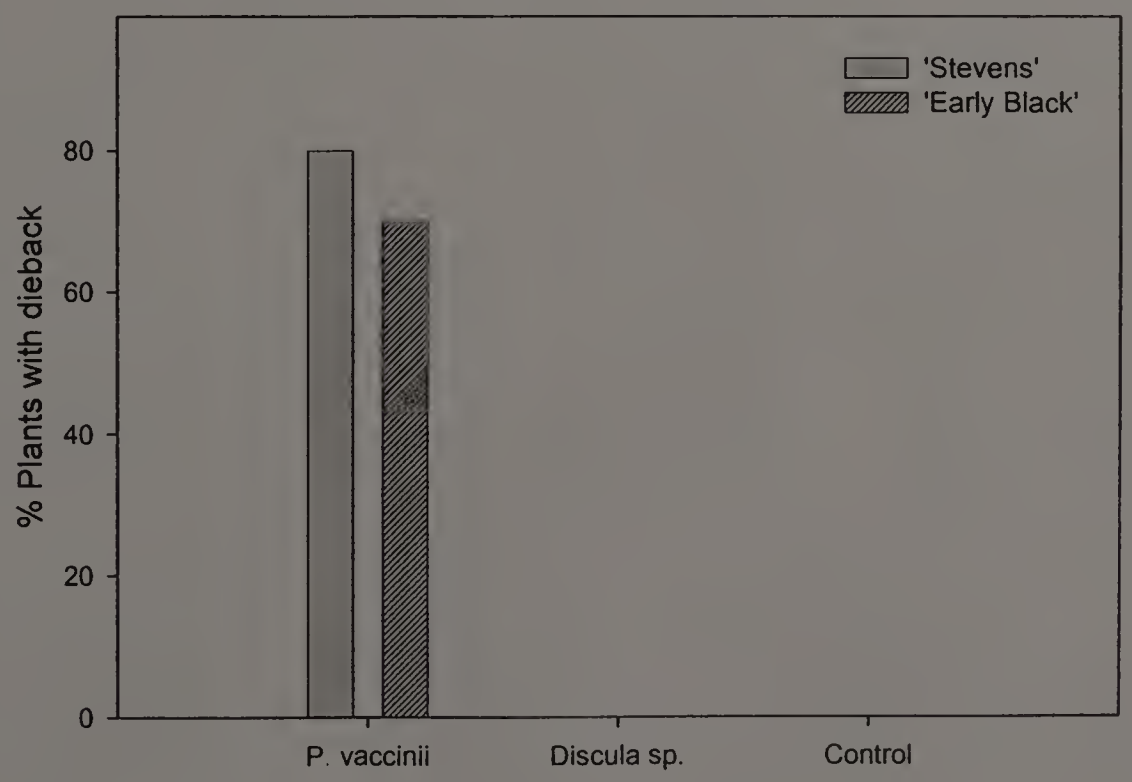


Figure 2.13. Percent of 'Stevens' and 'Early Black' rooted cuttings showing dieback symptoms after inoculation with agar plugs of *P. vaccinii*, *Discula* sp. or a sterile agar control (N=10).

Figures 2.14. 'Stevens' rooted cutting exhibiting symptoms 6 days (left) and 40 days (right) after wound-inoculation with *P. vaccinii*.

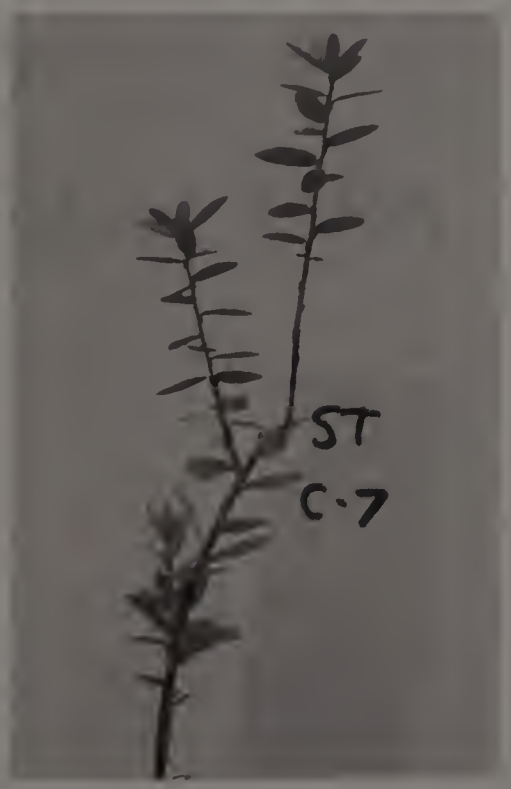
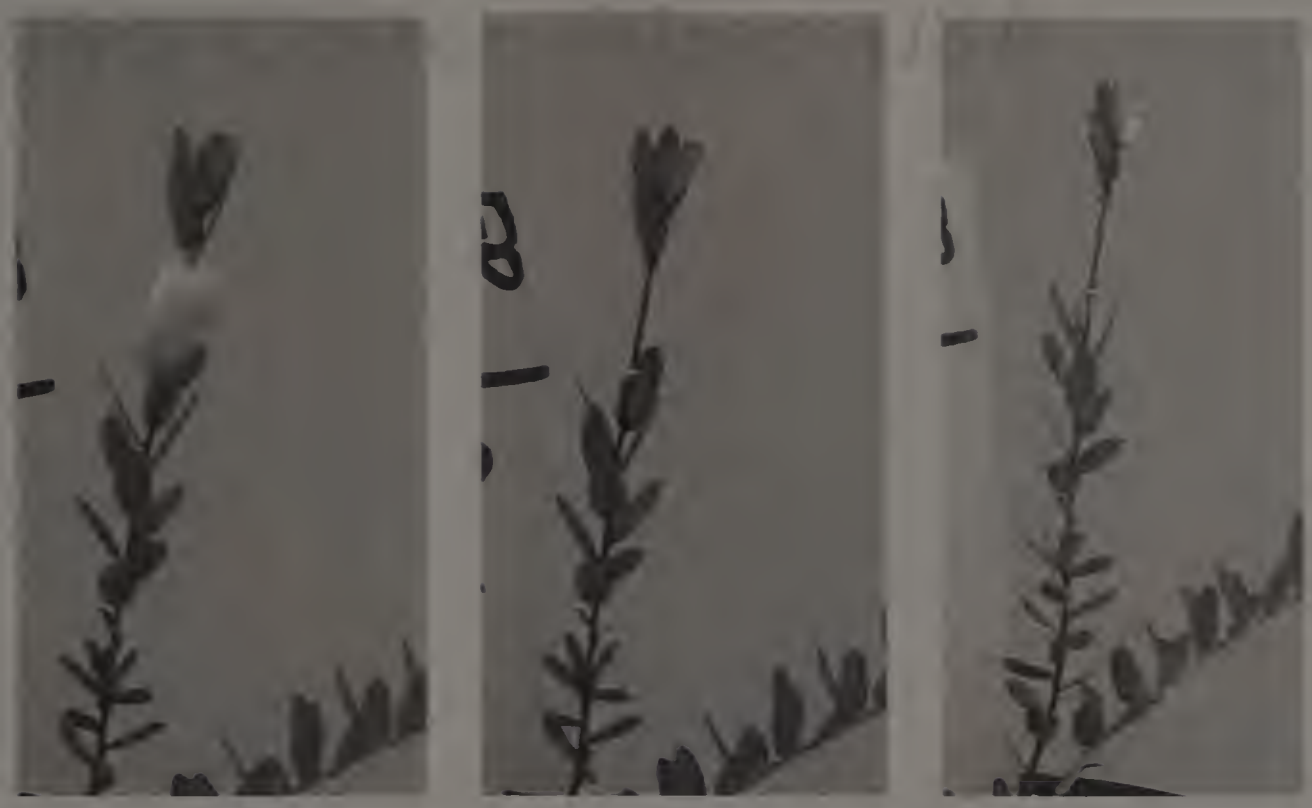


Figure 2.15. Healthy 'Stevens' rooted cutting 63 days after wound-inoculation with a sterile agar control.



Figures 2.16. 'Early Black' rooted cutting exhibiting symptoms 6 days (left), 10 days (center), and 14 days (right) after wound-inoculation with *P. vaccinii*.

Figure 2.17. Healthy 'Early Black' rooted cutting 64 days after wound-inoculation with a sterile agar control.

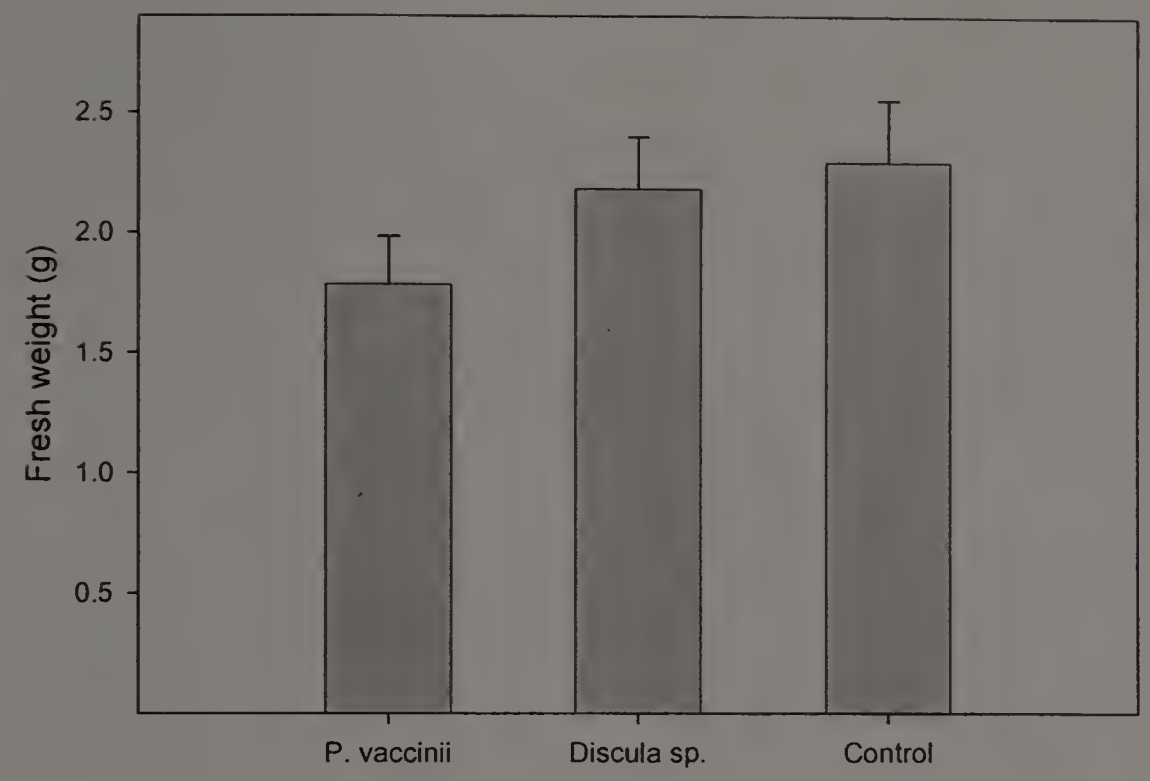


Figure 2.18. Fresh weight of 'Stevens' rooted cuttings approximately 12 months after inoculation with agar plugs of *P. vaccinii*, *Discula* sp. or a sterile agar control (N=10). Vertical bars represent standard error of means.

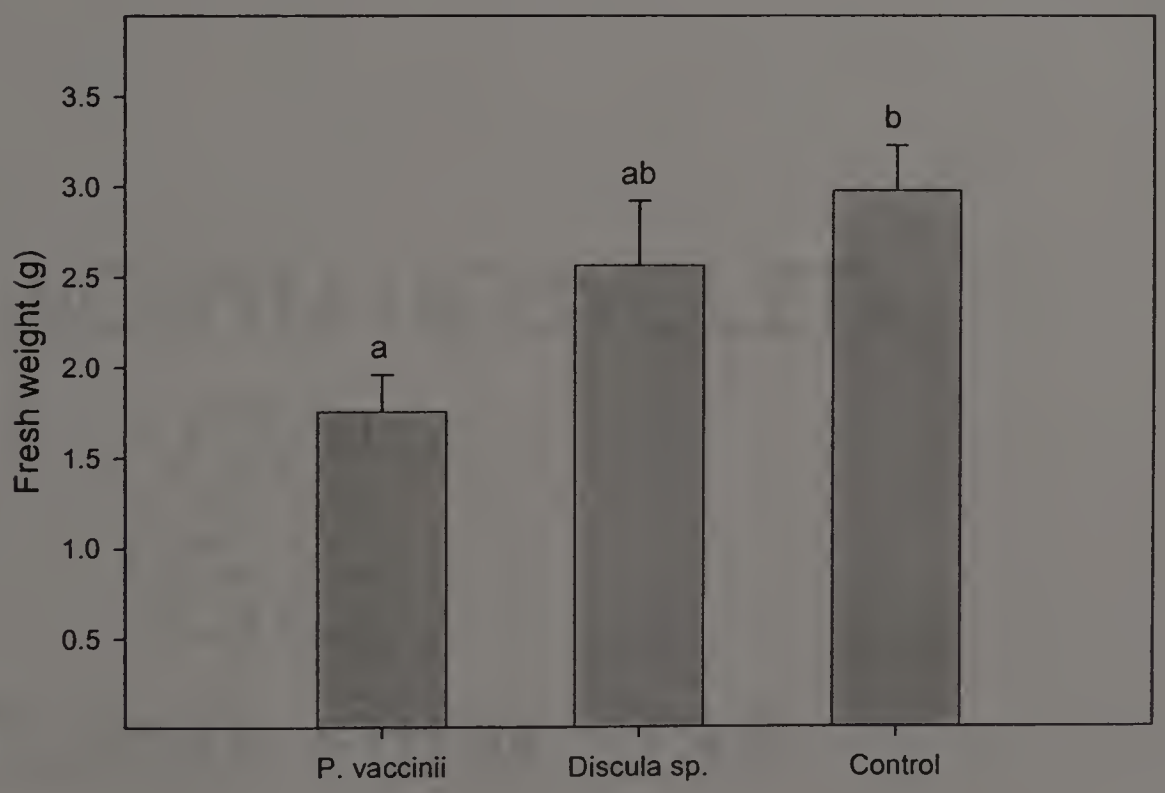


Figure 2.19. Fresh weight of 'Early Black' rooted cuttings approximately 10 months after inoculation with agar plugs of *P. vaccinii*, *Discula* sp. or a sterile agar control (N=10). Vertical bars represent standard error of means. Means with similar letters are not significantly different according to Kramer-adjusted Tukey's HSD (p = 0.05).

CHAPTER 3

INFECTION COURTS OF *PHOMOPSIS VACCINII*

Introduction

Koch's Postulates have been successfully completed for *Phomopsis vaccinii* on tissue-cultured cv. Early Black and cv. Stevens cranberry plants and rooted cuttings of the same cultivars, as described in Chapter 2. Due to the success of an early-spring application of a protective fungicide as a control measure for upright dieback, it has been assumed that infection occurs in the early spring on the new growth of the cranberry vines (Caruso and Ramsdell 1995). Numerous researchers have conducted inoculations of blueberry, *Vaccinium corymbosum*, and determined the function of wounding or stress in successful infection by *P. vaccinii*. Wilcox (1939) inoculated wounded and non-wounded blueberry plants with *P. vaccinii*, and noted that when woody tissue was inoculated, only localized lesions formed. Weingartner and Klos (1975) found similar symptoms when blueberry plants were wound-inoculated, but observed that infections occurring in the woody tissue expanded, killing the stem, and occasionally, the entire plant was killed by progression of the infection into the crown of the plant. Parker and Ramsdell (1977) determined that infection in Michigan required wounding, either abrasion or freezing injury. In North Carolina, though it was determined that infection occurred through wounded stem tissue and through leaf margins; infection occurred primarily through flower buds from budbreak through petal fall (Milholland 1982). In experiments to determine the most efficient inoculation

procedure to conduct resistance screening, Baker et al. (1995) determined that stem wounds resulted in the highest mortality.

The objective of this research was to determine the *P. vaccinii* sites of infection of cranberry and the importance of wounding in the infection process by inoculating different regions of the cranberry upright with different wounding techniques.

Materials and Methods

Rooted cuttings of cv. Stevens were inoculated with agar plugs (3 mm diameter) taken from the growing edges of a 3 to 5 day old colony of *P. vaccinii* maintained on half-strength potato dextrose agar (½PDA). The isolate of *P. vaccinii* used in this study, 98023, was also used in the proof-of-pathogenicity trials previously described in Chapter 2. Agar plugs of sterile ½ PDA were used to inoculate control plants.

Two regions of the cranberry upright were inoculated: the current-year growth – the new flush of growth of the current year's growth, and 1-yr-old growth – the woody tissue of the past-year's growth. Figure 3.1 shows a schematic of the inoculation treatments used in this study. Three wounding methods were used in the first experiment, and four were used in the second experiment. The wounding methods used were: (i) no wound, (ii) stem-pierce – the stem tissue was pierced once with a sterile needle (approximately 0.5 mm), (iii) leaf-scar – three to four leaves were removed, and (iv) leaf-pierce – three leaves were pierced three times each with a sterile needle (approximately 0.5 mm) (second experiment only).

Other than the various wounding methods, the same inoculation procedure used in the proof-of-pathogenicity trials using rooted cuttings was used for these experiments (Chapter 2). Plants were maintained in the greenhouse in a completely random design.

Observations were recorded every 7 to 14 days after inoculation for approximately 90 days in both trials. The first trial was initiated in the fall of 2002 with 5 replicate plants per treatment using plants that were rooted from cuttings in the spring of 2002, and the second trial was initiated in the spring of 2003 with 8 replicate plants per treatment using plants that were rooted from cuttings in the spring of 2002, maintained in the greenhouse through the growing season, and overwintered in a cooler.

At the termination of the second trial, the length of stem dieback was measured. Data were combined for each wound-tissue treatment group: leaf-pierce/current-year growth, leaf-pierce/1-yr-old growth, leaf-scar/current-year growth, leaf-scar/1-yr-old growth, no-wound/current-year growth, no-wound/1-yr-old growth, stem-pierce/current-year growth, and stem-pierce/1-yr-old growth. Any replicate with zero dieback was removed, and the remaining data were subjected to analysis of variance (ANOVA), and treatment means were separated using Kramer-adjusted Tukey's honestly significant difference (HSD) test (SAS V 9.1, SAS Institute).

Results

In the first trial, symptoms were only observed on inoculated plants when current-year growth was wounded by either the stem-pierce method or the leaf-scar method (Figure 3.2). No symptoms were observed when 1-yr-old growth or non-wounded tissue was inoculated. For the plants that exhibited dieback symptoms, all of the current-year's growth was affected, but tissue death did not progress into the past-year tissue (Table 3.1, Figure 3.4).

Similar to the first trial, symptoms were observed when wounded current-year growth was inoculated in the second trial. However, unlike the first trial, symptoms

were also observed on non-wound-inoculated current-year growth and on wound-inoculated 1-yr-old growth (Figure 3.3). Also unlike the first trial, different inoculation methods resulted in different symptom development (Table 3.1). For plants on which the current-year growth was inoculated with the stem-pierce method, the dieback symptoms affected all of the current-year growth, but did not progress into past-year's growth (Figure 3.5). For plants inoculated with the leaf-pierce, leaf-scar, or no-wound methods, only the portion of the upright above the point of inoculation was affected, and a new shoot often originated below the area of dieback (Figure 3.6). For plants on which the 1-yr-old growth was wounded with the stem-pierce or leaf-scar methods, dieback affected the entire upright above the point of inoculation but did not progress into uprights or runners adjoined to the inoculated upright (Figure 3.7).

Length of tip dieback in the second trial was significantly different between the wound-tissue treatments ($p < 0.0001$) (Figure 3.8). The length of dieback was the greatest for plants on which the 1-yr-old growth was inoculated with the leaf scar, 81.8 mm ± 0.0, and stem-pierce methods, 76.2 mm ± 13.6. Current-year growth inoculated with the stem-pierce method also resulted in a large amount of tissue death, 52.6 mm ± 6.3. Plants on which the current-year growth was inoculated with the leaf-pierce, leaf scar, or no-wound methods had less tissue death, 18.9 mm ± 6.6, 18.5 mm ± 1.1, and 24.0 mm ± 9.3, respectively.

Discussion

In the field, *P. vaccinii* infection is believed to occur in the early spring as uprights initiate growth (Caruso and Ramsdell 1995), and this suggestion is supported by this research. The young tissue of the new flush of growth was more susceptible to

infection by *P. vaccinii* than the woody tissue of the older growth. For both experiments, inoculated current-year growth resulted in a higher percent of inoculated plants developing dieback symptoms than inoculated 1-yr-old growth. In the first experiment, no plants developed dieback symptoms when the 1-yr-old growth was inoculated. In the second experiment, dieback symptoms were exhibited only when inoculated 1-yr-old growth was wounded.

Like *P. vaccinii* infection of blueberry, discussed above, *P. vaccinii* infection of cranberry can occur on non-wounded tissue though wounded tissue is more likely to manifest symptoms. In the first trial, only wound-inoculated current-year growth developed dieback symptoms, while a few of the non-wound-inoculated current-year growth developed dieback symptoms in the second trial. However, in these experiments, the early bud break and elongation stages were not inoculated. Young, succulent tissue, 3 to 4 cm from the tip of the upright, was inoculated. It is probable that this early elongation growth stage is highly susceptible to infection, and *P. vaccinii* infection could occur more frequently on this tissue with a non-wound inoculation than the results observed in this experiment. Although only a low percent of plants developed symptoms when inoculated on the 1-yr-old growth, a greater amount of tissue death occurred compared to the plants inoculated on the current-year-growth, as seen when the lengths of dieback were compared (Figure 3.8). This is due to the fact that the inoculation of 1-yr-old tissue occurred farther from the tip than the inoculation of current-year tissue. The stem-pierce method of inoculation resulted in the greatest amount of tissue death on inoculated current-year tissue compared to the other

inoculation methods. However this difference was only significant compared to the leaf-scar –inoculated current-year tissue.

The differences between the two trials should be noted. In the first trial only wound-inoculated current-year growth developed symptoms, while in the second trial non-wound-inoculated current-year growth and wound-inoculated 1-yr-old growth developed symptoms in addition to wound-inoculated current-year growth. Another difference between the first and second trials was the symptom development for the different wounding methods. In the first trial, the symptom development was the same for all the plants showing dieback symptoms – the current-year growth was killed, but the dieback did not progress into the 1-yr-old growth. In the second trial, different symptoms developed depending on the wounding method used. For current-year growth inoculated with the no-wound, leaf-pierce, or leaf-scar method, tissue desiccated to, but not past, the point of inoculation. For current-year growth inoculated with the stem-pierce method, tissue death progressed to, but not into, the past-year's growth.

The first trial was conducted in the fall of 2002 with cuttings that had been planted in the early spring of that year, and the second trial was conducted in the spring of 2003 with plants that had been planted in the early spring of the previous year and overwintered. The results from the first trial that indicate that infection can occur on the current-year's growth in the late season signifies that infection can occur, at least on wounded tissue, throughout the season. Previously, uprights were assumed to be infected during the early spring during bud break and early expansion and symptoms developed when suitable environmental conditions arose (Caruso and Ramsdell 1995). The environmental and plant stage differences might have been more conducive to

infection in the second trial, explaining the differences seen in the inoculated 1-yr-old growth and non-wounded tissue. However, in the first trial the leaf-scar method was more damaging than in the second trial. In the first trial, all of the current-year growth was killed, but in the second trial the current-year growth was only killed to the point of inoculation. The first trial was conducted in the fall and although the plants were maintained under lights to compensate for the shorter day length, these plants were not growing as vigorously as the plants of the second trial. This may be explained by a difference in the general health of the cuttings, or it may be possible that the resources available for plant defense vary with environmental factors or plant stage.

Only current-year growth was affected when infection occurs in the current-year growth, and infection did not progress to adjacent runners or uprights if the infection occurs in the 1-yr-old growth. The observation that dieback symptoms do not spread beyond the tissue in which the infection is initiated is consistent with observations in the field as well as observations from the proof-of-pathogenicity trials conducted with rooted-cuttings, discussed in Chapter 2. The results from this study indicate that older tissue can be infected if wounded, though infection will occur to a lesser extent than infection on current-year growth.

Current recommendations for upright dieback management include an early protective fungicide spray applied at bud break or early expansion, avoiding drought stress, and avoiding rank vine growth (Caruso 2005). The results from these studies that indicate that current-year growth is most susceptible support applying an early fungicide spray. Typically, one application of a protective fungicide is applied in the early season. Since, from these results, it appears that uprights are susceptible from bud

break through expansion and throughout the season on wounded tissue, it is questionable that one application in early spring will cover the infection period and sufficiently protect the plants from infection. Protective fungicides are also applied on a schedule during the flowering period for fruit-rot control (Oudemans et al. 1998), and these applications would also serve to protect the uprights. Considering the early spring spray and the fruit-rot sprays, a time period of susceptible growth is left unprotected before and after the fruit rot sprays. However, upright dieback is not usually a devastating disease, and it is not likely to be cost-effective to apply additional sprays to control this disease.

The information that wounding predisposes the cranberry plant to infection can also be considered as part of a management scheme. Avoiding pruning and unnecessary foot traffic on cranberry beds during periods of susceptibility, high moisture, and high inoculum presence would be prudent. Unfortunately, high inoculum periods of *P. vaccinii* for cranberry beds have not yet been well defined. Levesque (1999) determined that the detection of *P. vaccinii* spores reached a peak in mid- to late-May on two cranberry beds in British Columbia. In blueberry fields, conidia of *P. vaccinii* were present during rains from bloom through petal fall and, to a lesser extent, during rains later in the season in Michigan (Parker and Ramsdell 1977), and conidia were collected from late February to early August in North Carolina (Milholland 1982). Warm temperatures, 21 to 27 °C, are most favorable for germination and growth of *P. vaccinii*, and have been suggested to be a factor in determining the period of inoculum presence and infection period for both blueberry and cranberry (Wilcox 1939, Parker and Ramsdell 1977, Friend and Boone 1968).

Due to the involvement of wounds from to freezing injury in *P. vaccinii* infection of blueberry (Parker and Ramsdell 1977), it is reasonable to consider that freezing injury may also be involved in predisposing cranberry plants to infection. Thus, protecting vines during frosts in the spring could help reduce infection. However, there is anecdotal evidence that excessive leaf wetness in the spring predisposes cranberry uprights to upright dieback, therefore prudent use of water during this time period is recommended until further research can better determine the environmental factors involved, the periods of high inoculum, and the infection period in the field.

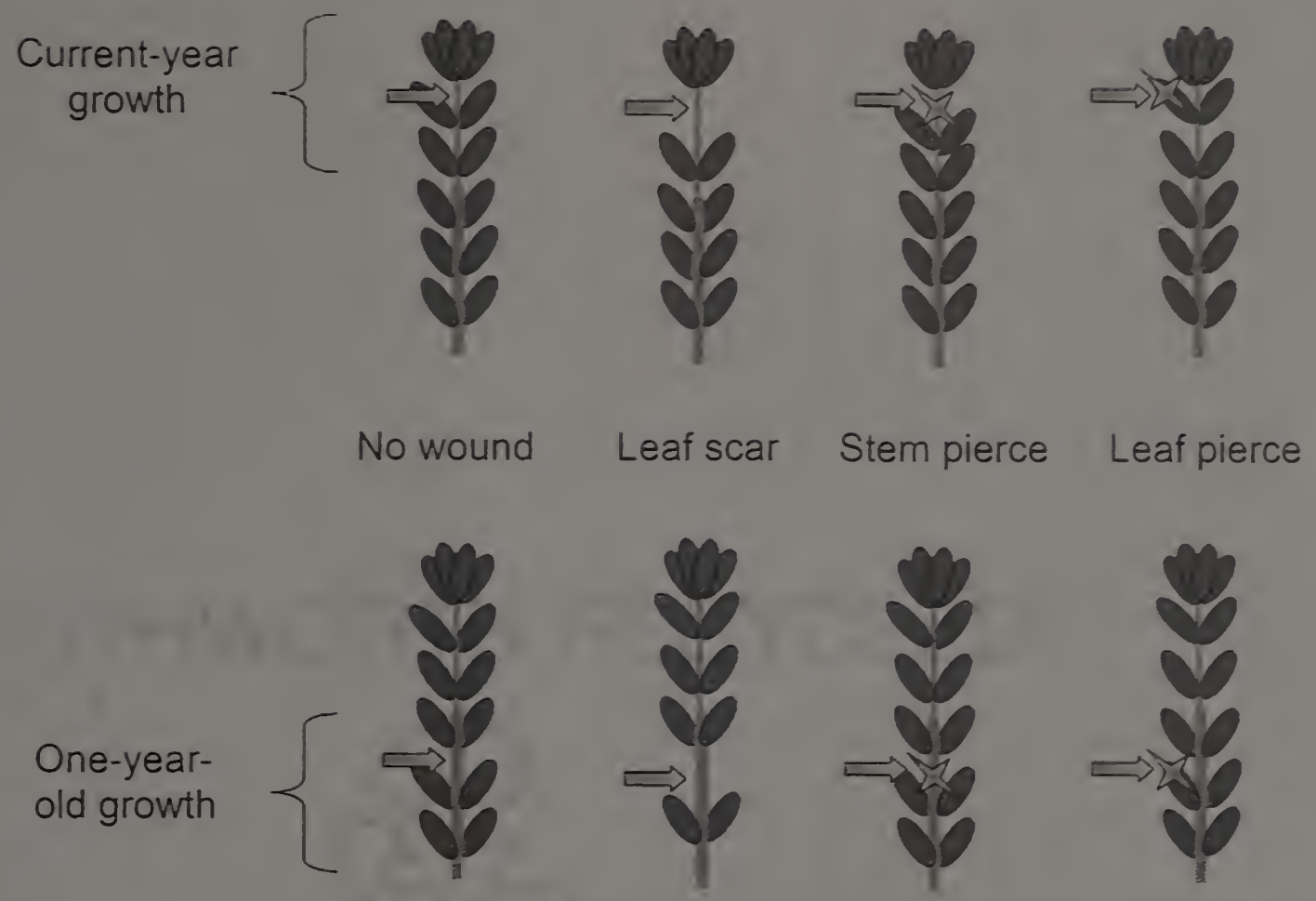


Figure 3.1. Schematic of the inoculation procedures used in these experiments. Two tissue types (current-year growth and one-yr-old growth) were inoculated using different wounding techniques. (No wound: the plants were not wounded. Leaf scar: 3 to 4 four leaves were removed. Stem pierce: the stem tissue pierced once with a sterile needle. Leaf pierce: 3 leaves were pierced 3 times each with a sterile needle. The leaf-pierce technique was only used in Experiment 2). Arrow indicates the point of inoculation.

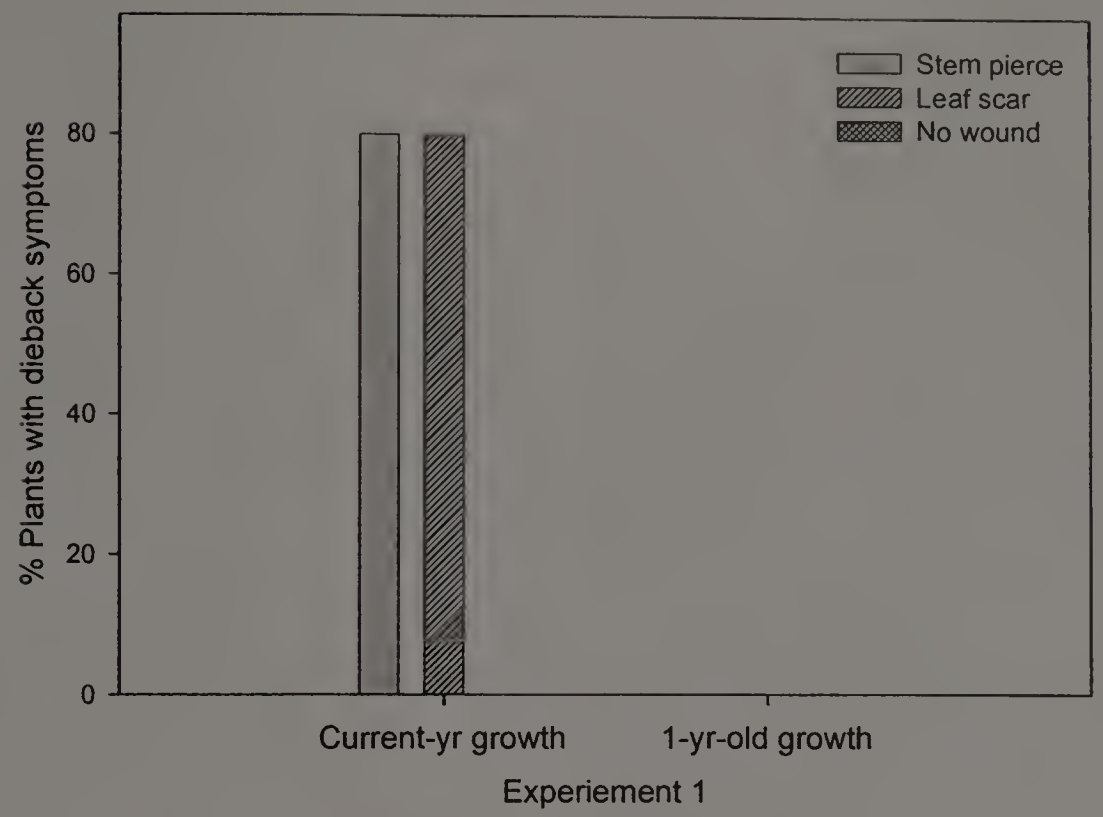


Figure 3.2. Percent of 'Stevens' plants showing upright dieback symptoms after inoculation with *P. vaccinii* when different regions of the upright were inoculated using different wounding methods (Experiment 1: N=5).

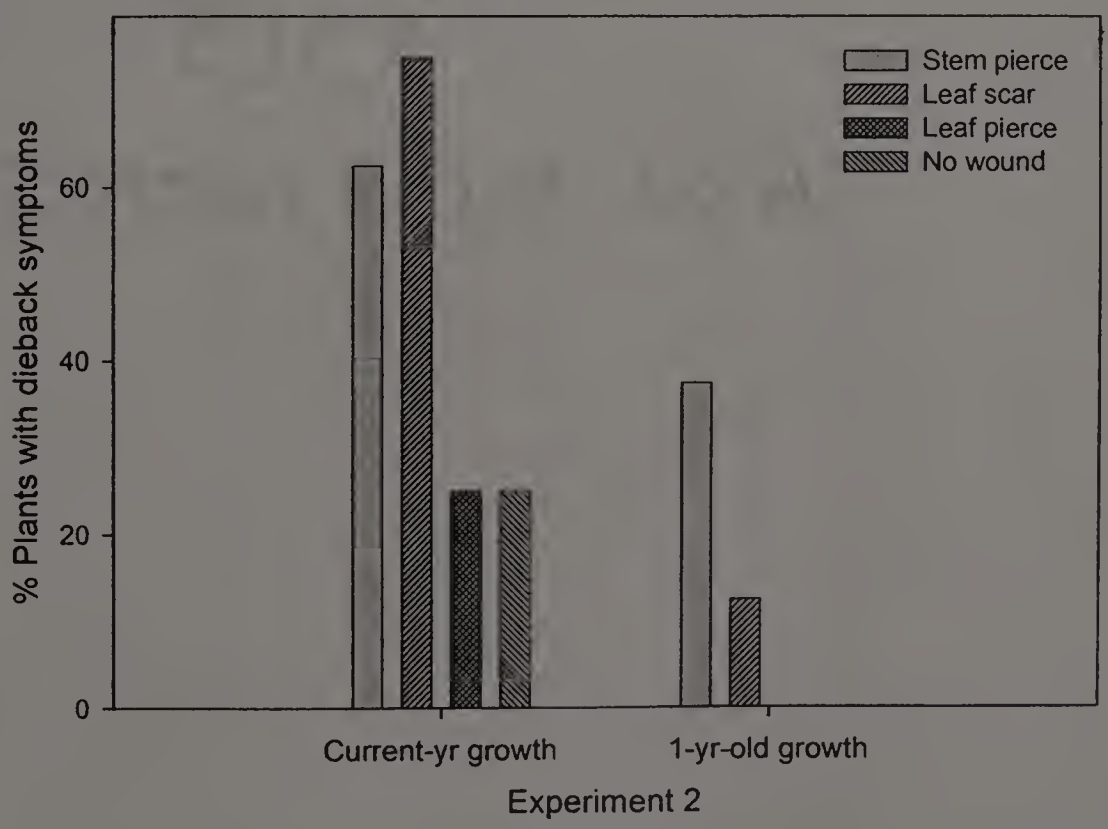


Figure 3.3. Percent of 'Stevens' plants showing upright dieback symptoms after inoculation with *P. vaccinii* when different regions of the upright were inoculated using different wounding methods (Experiment 2: N=8).

Table 3.1. Description of the symptom development of 'Stevens' plants after inoculation with *P. vaccinii* when different regions of the upright were inoculated using different wounding methods.

Tissue Type	Wound Type	Experiment 1	Experiment 2
Current-year growth	No wound	No symptoms	Dieback, no desiccation beyond the point of inoculation. A new shoot often originated below the area of dieback.
	Stem pierce	Dieback, killing current-year growth No progression into older growth.	Dieback, killing current-year growth. No progression into older growth.
	Leaf scar	Dieback, killing current-year growth. No progression into older growth.	Dieback, no desiccation beyond the point of inoculation. A new shoot often originated below the area of dieback.
	Leaf pierce	N/A	Dieback, no desiccation beyond the point of inoculation. A new shoot often originated below the area of dieback.
One-year-old growth	No wound	No symptoms	No symptoms
	Stem pierce	No symptoms	Dieback affecting entire upright above point of inoculation. No progression into adjoined uprights or runners.
	Leaf scar	No symptoms	Dieback affecting entire upright above point of inoculation. No progression into adjoined uprights or runners.
	Leaf pierce	N/A	No symptoms

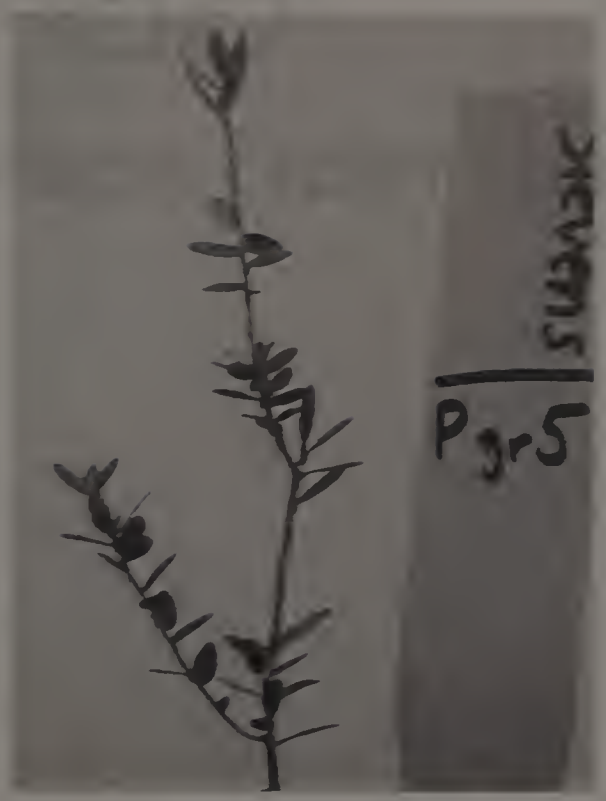


Figure 3.4. Symptoms observed on plants on which the current-year growth was wound-inoculated with *P. vaccinii* using a leaf-scar or stem-pierce technique (Experiment 1). Dieback symptoms progressed beyond the point of inoculation, but affected the current-year growth only.

Figure 3.5. Symptoms observed on plants on which the current-year growth was inoculated with *P. vaccinii* using a stem-pierce technique (Experiment 2). Dieback symptoms progressed beyond the point of inoculation, but affected the current-year growth only.

Figure 3.6. Symptoms observed on plants on which the current-year growth was inoculated with *P. vaccinii* using a no-wound technique, leaf-scar technique, or leaf-pierce technique (Experiment 2). Tissue desiccation did progress beyond the point of inoculation and, in many cases a new shoot formed below the necrotic portion of the upright.

Figure 3.7. Symptoms observed on plants on which the one-year-old growth was wound-inoculated with *P. vaccinii* using either a leaf-scar or stem-pierce technique (Experiment 2). The entire upright was affected, but the symptoms did not progress into uprights adjoined to the inoculated upright.

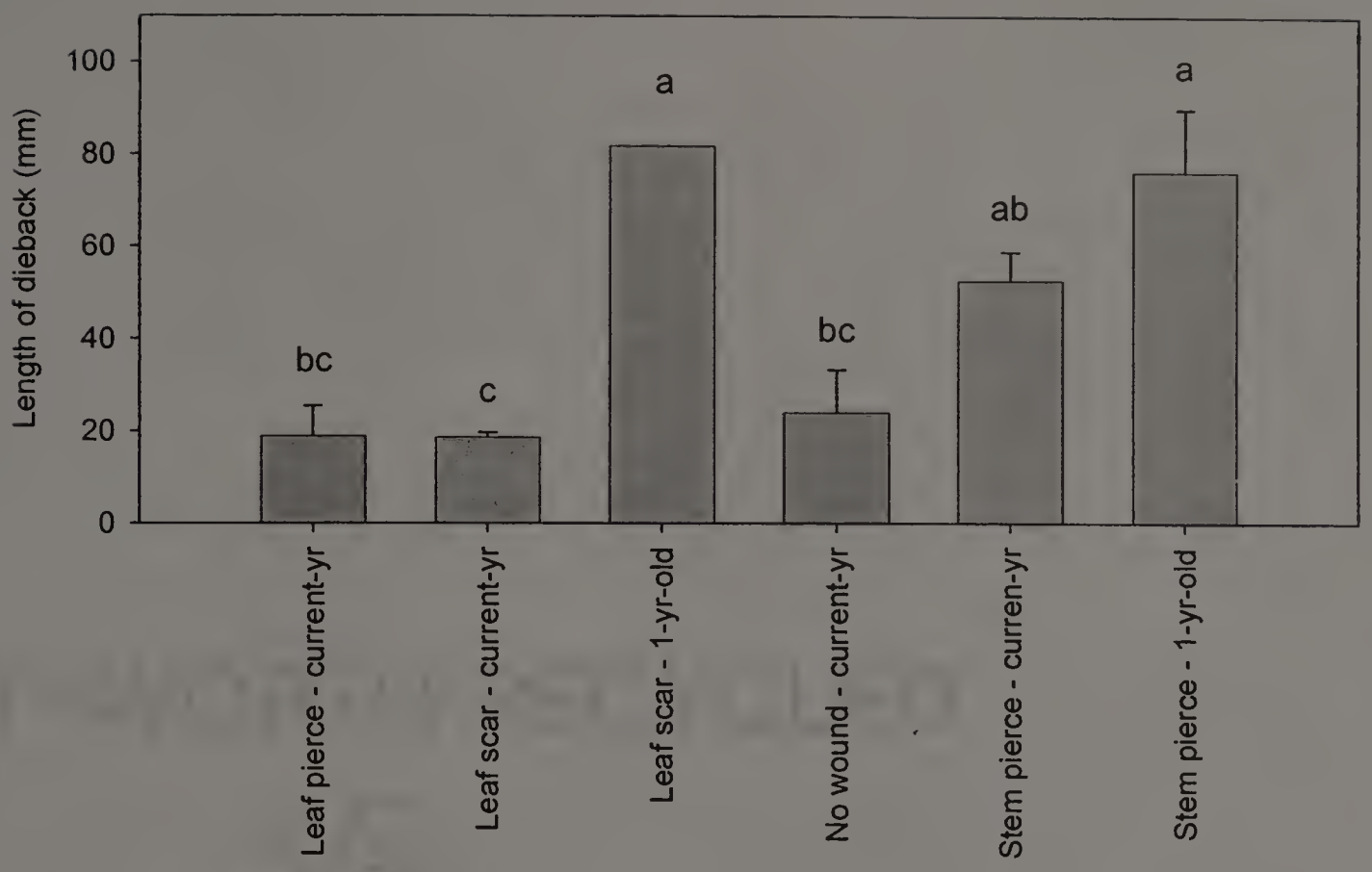


Figure 3.8. Length of tip dieback of only replicates showing disease symptoms after inoculation of different regions of cranberry uprights with *P. vaccinii* using different wounding techniques in Experiment 2 (leaf-pierce/current-yr: N=2, leaf scar/current-yr: N=6, leaf-scar/1-yr-old: N=1, no-wound/current-yr: N=2, stem-pierce/current-yr: N=5, stem-pierce/1-yr-old: N=3). Vertical bars represent standard error of the means. Means with similar letters are not significantly different (p = 0.05).

CHAPTER 4

PHOMOPSIS VACCINII AND *PHOMOPSIS* SP. PATHOGENICITY TESTS

Introduction

As previously reported in Chapter 2, *Phomopsis vaccinii* was determined to be a causal agent of upright dieback disease of cranberry, *Vaccinium macrocarpon*. *P. vaccinii* is also associated with fruit rot of cranberry and highbush blueberry, *Vaccinium corymbosum* (Shear 1931, Milholland and Daykin 1983, Caruso and Ramsdell 1995), and stem and canker diseases of blueberry (Wilcox 1939, Weingartner and Klos 1975, Parker and Ramsdell 1977, and Milholland 1982).

Species included in the genus *Phomopsis* are predominantly described on the basis of plant host (Uecker 1988). However, there is recent evidence that identification of fungi in the genus *Phomopsis* is a more complicated endeavor. Rehner and Uecker (1994) examined numerous isolates of *Phomopsis* from a wide range of plant hosts. After phylogenetic analysis of two internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA (rDNA), little association was found between the host of isolation and the groups of *Phomopsis* as determined by the ITS sequence analysis. Farr et al. (2002) characterized numerous isolates of *Phomopsis* from highbush blueberry and cranberry by comparing sequences of ITS rDNA as well as morphological characteristics. While most isolates grouped with an authentic culture of *P. vaccinii*, some isolates were determined to be related to *Phomopsis* isolates from diverse hosts and thus were excluded from *P. vaccinii*.

P. vaccinii is the anamorph of *Diaporthe vaccinii*, and both fungi were originally described by Shear et al (1931). Other than the original description and a report by Wilcox (1940), no reports of the *Diaporthe* teleomorph exist. Due to the absence of teleomorph specimens that can be associated with *P. vaccinii*, Farr et al (2002) ignored the *D. vaccinii* name and applied *P. vaccinii* to all isolates with the caveat that the *Diaporthe* name would apply if the *Diaporthe* state were found on a *Vaccinium* host and the isolates were shown to agree with *P. vaccinii*.

Some isolates used in various blueberry inoculation studies were included in the analysis by Farr et al. (2002). An isolate used in the studies by Weingartner and Klos (1975) and an isolate used in the experiments conducted by Chao and Glawe (1985) were both determined by Farr et al. to be included in *P. vaccinii*. Also, the *P. vaccinii* isolate that was used in the proof-of-pathogenicity trials and the infection-court trials, presented earlier in this dissertation (Chapters 2 and 3), was determined by Farr et al. to be *P. vaccinii*. However, other isolates of *P. vaccinii* and *Phomopsis* sp. from blueberry and cranberry have not been tested for pathogenicity. The objective of this study was to determine the pathogenicity of a variety of isolates identified as *P. vaccinii* and *Phomopsis* sp. that were isolated from highbush blueberry and cranberry plants.

Materials and Methods

Blueberry Rooted Cuttings

The cultivar Bluejay was chosen for inoculation trials based on a study by Baker et al. (1995), which determined that this cultivar was one of the most susceptible cultivars of those tested. In 2001, 1-year-old 'Bluejay' rooted microshoots were

transferred to 12.7 cm pots, maintained in greenhouse conditions, and used for two inoculation trials. The first trial was initiated in June 2001, and the second trial was initiated in June 2002.

The same inoculation procedure described for cranberry rooted cuttings in Chapter 2 was used in these studies, and the fungi used in the blueberry inoculations are listed in Table 4.1. Each isolate treatment was replicated 7 times. One branch on one plant was considered a replicate. Observations were recorded throughout the summer for the first trial, after which the plants were overwintered and observed again the following summer. Observations for the second trial were recorded only for the summer after inoculation. Any replicate plants that were adversely affected by other factors, such as heat stress, were removed from the experiment.

At the termination of the experiment, stem samples of each treatment were taken near the point of inoculation. The small sections of plant tissue were surface sterilized by soaking for 15 s in 70% ethanol (ETOH) followed by 2 min in a 0.5% sodium hypochlorite (NaOCl) solution plus Tween80®. The stem sections were plated onto half-strength acidified potato dextrose agar (½ APDA) and observed for fungal growth after approximately 3 weeks of incubation at room temperature.

Tissue-cultured Cranberry Plants

Inoculations of tissue-cultured plants were conducted with both cv. Stevens and cv. Early Black. The origin and maintenance of the plant tissue cultures were as described in Chapter 2. Plugs of agar and mycelium were used as inoculum, and sterile agar plugs were used in controls, also as described in Chapter 2. The fungi used for these inoculations are listed in Table 4.2. The no-wound procedure of inoculation, also

described in Chapter 2, was used in these studies. Observations were recorded weekly for 50 days for both the 'Stevens' and the 'Early Black' trials. Each treatment was replicated 8 times in both trials. Any replicate in which the plant growing media became contaminated with fungi or bacteria was removed from the experiment.

At the termination of the experiments, a small portion of tissue was selected near the point of inoculation, surface disinfested by a 15-s soak in 70% ETOH, plated on ½ APDA, and observed for fungal growth after 2 to 3 weeks of incubation at room temperature. Additionally, dry shoot weight was recorded, and the data subjected to analysis of variance (ANOVA) (SAS V 9.1, SAS Institute). Treatment means were separated using Kramer-adjusted Tukey's honestly significant difference (HSD) test. To better meet the model assumptions of ANOVA, the 'Early Black' data were first square-root transformed.

Cranberry Rooted Cuttings

Cuttings taken from an 'Early Black' bed at the University of Massachusetts Cranberry Station, East Wareham, MA were rooted in the early spring of 2004 and maintained in greenhouse conditions. These plants were inoculated in July 2004.

Plugs of agar and mycelium as previously described (Chapter 2) were used as inoculum, and sterile agar plugs were used for controls. The same isolates that were used for the tissue-culture inoculations discussed above were used in this greenhouse trial (Table 4.2). The same inoculation procedure discussed in the proof-of-pathogenicity trials conducted with rooted cuttings of cranberries (Chapter 2) was used in these experiments. Each treatment was replicated 9 times. One upright on one cranberry plant was considered a replicate.

Any replicate plant that was obviously affected by factors other than the inoculum, such as heat stress or physical damage, was removed from the experiment. After inoculation, observations were recorded every 7 days for approximately 60 days. At the termination of the experiment, the length of dieback for each plant was measured and recorded. The data from the replicates that resulted in symptom development were subjected to ANOVA, and treatment means separated using Kramer-adjusted Tukey's HSD test (SAS V 9.1, SAS Institute). Additionally, small sections of tissue were sampled at the point of inoculation from each treatment. The stem samples were surface sterilized using the same procedure as the blueberry stem samples as described above, plated on ½ APDA, and observed for fungal growth after 2 to 3 weeks of incubation at room temperature.

Results

Blueberry Rooted Cuttings

Dieback Symptoms

Inoculation with *P. vaccinii* isolates from blueberry and cranberry resulted in canker or dieback symptoms, while inoculation with any *Discula* sp. isolate did not result in symptoms. Occasionally, on otherwise symptomless plants, raised tissue was observed around the inoculation wounds on plants inoculated with isolates of *P. vaccinii*, *Discula* sp., and control plants. For the two trials conducted, 57.1% ± 0.0 of the plants developed symptoms when inoculated with *P. vaccinii* isolate 93020A, 64.3% ± 7.1 of plants developed symptoms when inoculated with *P. vaccinii* isolate 98021,

and 35.7% ± 7.1 of plants developed symptoms when inoculated with *P. vaccinii* isolate 98023 (Figure 4.1, Table 4.3).

Recovery of fungi

P. vaccinii isolates 93020A and 98023 were only recovered from diseased plants, while *P. vaccinii* isolate 98021 was recovered from both diseased and symptomless plants. No pathogenic fungi were isolated from control plants, and *Discula* sp. was not isolated from plants inoculated with any of the *Discula* sp. isolates (93009C, 93011B, 93012B, or 98020).

Tissue-cultured Cranberry Plants

Dieback Symptoms

Of the 'Early Black' plants inoculated, *P. vaccinii* isolates 93020A, 98023, ATCC 18451, and DF 5022 produced dieback symptoms on 62.5%, 50.0%, 12.5%, and 12.5% of the inoculated plants, respectively (Figure 4.2, Table 4.3). *Phomopsis* sp. isolates DF 5025 and DF 5036 also produced symptoms on inoculated 'Early Black' plants, both isolates affecting 28.6% of the plants inoculated. No symptoms were observed on any 'Early Black' control plants.

On the inoculated 'Stevens' plants, *P. vaccinii* isolates 93020A and 98023 produced dieback symptoms, both affecting 62.5% of the plants inoculated (Figure 4.2, Table 4.3). *Phomopsis* sp. isolate DF 5036 produced dieback symptoms on 12.5% of the plants inoculated. No 'Stevens' control plants showed dieback symptoms.

The following isolates did not result in disease development on either 'Early Black' or 'Stevens' tissue-cultured plants: *P. vaccinii* isolates CBS 160.32, DF 5044, and *Phomopsis* sp. isolates ATCC 56789, DF 5041, FAU 445, and FAU 453.

Dry weight

Although there were no significant differences between the dry weights of the 'Early Black' plants inoculated with the various isolates, isolates that resulted in symptoms were among the treatments with the lowest dry weights (Figure 4.3). Dry weights were significantly different among the isolates in the 'Stevens' trial ($p < 0.0001$). Generally, the average dry weight of the 'Stevens' plants inoculated with isolates that resulted in symptom development was less than the dry weight of plants inoculated with isolates that did not result in symptom development (Figure 4.4). However, only isolates 93020A (59.0 mg ± 8.0), DF 5044 (61.0 mg ± 5.7), and DF 5036 (43.5 mg ± 8.8), had dry weights that were significantly different from the dry weight of the control (103.4 mg ± 7.2). Isolates 93020A and DF 5036 resulted in symptom development; however, isolate DF 5044 did not result in symptom development.

Recovery of fungi

All inoculated-type *P. vaccinii* or *Phomopsis* sp. were recovered from samples of diseased 'Early Black' and 'Stevens' plants. Other than *P. vaccinii* isolate DF 5022, all isolates of *Phomopsis* sp. and *P. vaccinii* were isolated from samples of symptomless 'Early Black' plants. All isolates of *Phomopsis* sp. and *P. vaccinii* were isolated from samples of symptomless 'Stevens' plants, except *P. vaccinii* isolates 98023 and DF

5022 and *Phomopsis* sp. isolate DF 5036 . No fungi were isolated from any of the control replicates in either the 'Early Black' trial or the 'Stevens' trial.

Cranberry Rooted Cuttings

Dieback Symptoms

P. vaccinii isolates 93020A, 98023, ATCC 18451, CBS 160.32 and DF 5022 produced symptoms on inoculated 'Early Black' rooted cuttings (Figure 4.5, Table 4.3). Isolate 93020A produced symptoms on 100% of plants inoculated. With isolates 98023, ATCC 18451, and DF 5022, 88.9% of inoculated plants exhibited dieback symptoms. Of the plants inoculated with *P. vaccinii* isolate CBS 160.32, 33.3% showed dieback symptoms. *Phomopsis* sp. isolates DF 5025, DF 5036, and DF 5041 produced symptoms on 11.1%, 33.3%, and 33.3% of inoculated plants, respectively. Control plants, plants inoculated with *P. vaccinii* isolate DF 5044, and plants inoculated with *Phomopsis* sp. isolates FAU 453, FAU 445, and ATCC 56789 did not exhibit any dieback symptoms or response to infection.

Length of Dieback

The average length of dieback on the inoculated rooted cuttings was not significantly different among the isolates tested ($p = 0.5049$). Plants inoculated with *P. vaccinii* isolates, 93020A, 98023, ATCC 18451,CBS 160.32, and DF 5022 resulted in 32.2 mm ± 1.6, 35.6 mm ± 3.1, 37.7 mm ± 4.5, and 33.3 mm ± 2.0, respectively (Figure 4.6). Plants inoculated with *Phomopsis* sp. isolates DF 5025, DF 5036, and DF 5041 resulted in 27.8 mm ± 0.0, 31.6 mm ± 4.0, and 42.1 mm ± 0.0, respectively.

Recovery of fungi

Of the isolates that resulted in infection and symptom development, all *Phomopsis* sp. isolates were recovered from plated diseased tissue samples, and all *P. vaccinii* isolates were recovered from plated diseased tissue samples, except ATCC 18451. Additionally, *P. vaccinii* was recovered from symptomless tissue inoculated with 93020A, 98023, and CBS 160.32, and *Phomopsis* sp. was recovered from symptomless tissue samples of DF 5041, FAU 453, FAU 445, ATCC 56789, DF 5025, and DF 5036. *P. vaccinii* or *Phomopsis* sp. were not recovered from control plants, or from any plants inoculated with ATCC 18451, DF 5044, or from symptomless plants inoculated with DF 5022.

Discussion

P. vaccinii, *Phomopsis* sp., and *Discula* sp. pathogenicity

All three of the *P. vaccinii* isolates used in the blueberry trial (93020A, 98021 and 98023) resulted in symptom development on the inoculated 'Bluejay' blueberry plants. Unfortunately, since many of the desired isolates had not been identified or available prior to the inception of the blueberry inoculations, all but two of the isolates used in the blueberry trials were different from the isolates used in the tissue-cultured cranberry trials and the cranberry rooted-cutting trial. *P. vaccinii* isolates, 93020A and 98023 were used in both the blueberry and cranberry trials. These two isolates also resulted in dieback symptoms on inoculated 'Early Black' and 'Stevens' tissue-cultured cranberry plants and inoculated 'Early Black' cranberry rooted cuttings.

As mentioned above, *P. vaccinii* isolates 93020A and 98023 resulted in disease in inoculated 'Bluejay' blueberry rooted cuttings, 'Stevens' and 'Early Black' tissue-cultured cranberry plants, and 'Early Black' cranberry rooted cuttings. Additional *P. vaccinii* isolates, ATCC 18451 and DF 5022, resulted in dieback symptoms on tissue-cultured 'Early Black' plants as well as 'Early Black' rooted cuttings,. *P. vaccinii* isolate CBS 160.32 only resulted in dieback on inoculated 'Early Black' cranberry rooted cuttings. It was observed that all *P. vaccinii* isolates caused symptom development on cranberry rooted cuttings, except DF 5044. This was isolated in 1999, and this result can possibly be attributed to loss of characteristics after a long period of storage. However, older isolates remained pathogenic, for example isolate 93020A, which consistently caused symptom development on inoculated plants, was isolated in 1993, and CBS 160.32, which resulted in symptom development on cranberry rooted cuttings, was isolated in 1932. Isolate DF 5044 was the only *P. vaccinii* isolate from blueberry fruit that was tested. While it may be possible that fruit isolates will not cause a stem infection, this conclusion is not justified since only one blueberry fruit isolate was tested, and no cranberry fruit isolates were tested. Also, although few inoculation studies have been conducted with isolates from fruit, Wilcox (1939) determined that *P. vaccinii* isolates from rotted cranberry fruits were responsible for stem infections of inoculated blueberry plants.

As determined by the analysis completed by Farr et al. (2002), some *P. vaccinii* isolates used in this study had identical ITS sequences: *P. vaccinii* isolates 93020A, 98021, 98023, DF 5044, and DF 5022. Also, *P. vaccinii* isolates ATCC 18451 and CBS 160.32 were found to have identical ITS sequences. While it is not expected that

isolates with identical ITS sequences will have identical attributes, it is of interest to compare the similar isolates. While most of the isolates listed above in the first group of isolates with identical ITS sequences resulted in relatively similar symptom development, DF 5044 did not result in disease development on any plant inoculated in the various trials. Both isolates of the second group with the same ITS sequences, ATCC 18451 and CBS 160.32, resulted in disease development.

Isolates of *Phomopsis* sp. also resulted in dieback symptoms on inoculated tissue-cultured cranberry plants and cranberry rooted cuttings. Some *Phomopsis* sp. isolates – FAU 453 and the isolates that are reported to group with *Diaporthe eres*, ATCC 56789 and FAU 445 (Castlebury) – did not result in symptom development on any cranberry plant in any of the trials. Other *Phomopsis* sp. isolates, DF 5025, DF 5036, and DF 5041, resulted in a low percent of inoculated plants developing symptoms in one or more trials. Although the *Phomopsis* sp. isolates were all isolated from either blueberry or cranberry, the isolates are not closely related, as determined by Farr et al. (2002), and these *Phomopsis* sp. isolates group with *Phomopsis* isolates from a wide range of hosts. While these isolates are discussed as a group, this grouping is artificial for the purpose of a comparison of the *P. vaccinii* isolates verses the non-*P. vaccinii* *Phomopsis* sp. isolates.

It is worthwhile to draw the conclusion that *P. vaccinii* isolated from blueberry can infect cranberry and vice versa. In these studies, isolates of *P. vaccinii* from both cranberry and blueberry resulted in disease development on blueberry rooted microshoots, tissue-cultured cranberry plants, and rooted cuttings of cranberry. Wilcox (1939) also reproduced symptoms on blueberry plants with isolates from both cranberry

and blueberry. Additionally, *Phomopsis* sp. isolates from blueberry resulted in symptom development on tissue-cultured cranberry plants and cranberry rooted cuttings.

Dry weights were measured at the termination of both of the tissue-cultured trials. In the previously discussed proof-of-pathogenicity trials, dry weight of isolate treatments allowed for a comparison of relative pathogenicity (Chapter 2). In these studies, however, a general trend can be observed that the dry weight of the isolate treatments that resulted in symptom development was less than the dry weight of isolate treatments that remained symptomless, but there were few clear differences. No significant differences were found between the dry weights of the isolate treatments in the 'Early Black' tissue-cultured cranberry plants. In the 'Stevens' trial, there were significant differences between isolate treatments, though only isolates 93020A, DF 5036, and DF 5044 had dry weights different from the control. However, inoculation with isolate DF 5044 did not result in symptom development, and isolate 98023, which resulted in one of the highest number of affected plants (62.5% in the 'Stevens' trial), did not have an average dry weight that was different from the control or other isolate treatments that did not result in symptom development. It can be concluded from this study that, unlike the proof-of-pathogenicity trials conducted with tissue-cultured plants discussed earlier (Chapter 2), dry weight was not a good indicator of relative pathogenicity. Tissue-cultured plants were maintained in a controlled environment, and it was not expected that the environmental conditions would affect the trials. However, tissue-cultured plants are sensitive to environmental fluctuations and it is possible that conditions in the growing room varied slightly and affected plant growth in the various

trials. In these isolate tests, the plant size was not as uniform at the initiation of the experiments as in the proof-of-pathogenicity trials (Chapter 2). Though an effort was made to evenly distribute the various sizes of plants across all treatments, this size differential may have made differences more difficult to discern statistically.

There were no significant differences among the lengths of dieback measured at the termination of the cranberry-rooted-cutting trial. While the percent of plants that developed symptoms after inoculation varied among the isolates, the amount of tissue death was consistent when symptoms developed.

When considering all the trials, *P. vaccinii* isolates were more pathogenic than *Phomopsis* sp. isolates. More *P. vaccinii* isolates resulted in disease development of inoculated plants than *Phomopsis* sp. isolates. *P. vaccinii* isolates together resulted in 22.2% of inoculated 'Early Black' tissue-cultured plants and 21.3% of inoculated 'Stevens' tissue-cultured plants showing dieback symptoms, while *Phomopsis* sp. isolates together resulted in 9.1% of inoculated 'Early Black' tissue-cultured plants and 2.2% of 'Stevens' tissue-cultured plants showing symptoms. On inoculated cranberry rooted cuttings, *P. vaccinii* isolates together resulted in 66.7% of inoculated plants developing symptoms, and *Phomopsis* sp. isolates resulted in only 9.4% of inoculated plants developing symptoms.

As expected, most *P. vaccinii* and *Phomopsis* sp. isolates that resulted in disease development on inoculated plants were recovered from diseased tissue. In addition, *P. vaccinii* and *Phomopsis* sp. isolates were isolated from plants that remained symptomless. Numerous researchers have reported the culture of *Phomopsis* from symptomless cranberry stems and fruits (Boone 1968, Stiles and Oudemans 1999,

Catlin and Caruso 2001). This frequent recovery from symptomless tissue might be explained by a latent infection or an endophytic stage. Farr et al. (2002) also suggested the possibility that the isolates of *Phomopsis* isolated from blueberry and cranberry that were determined not to be *P. vaccinii* may be endophytes. Finding that species of *Phomopsis* have endophytic relationships with cranberry would not be surprising. Endophytic fungi routinely are found in woody plants, and researchers frequently have found that endophytes are often closely related to pathogens of the same host or a closely related host (Saikkonen et al. 1998). Additionally, species of *Phomopsis* have been reported to have endophytic relationships with various woody plants including Ericaceous plants (Petrini 1984), Japanese beech (Sahashi et al. 1999), redwood (Espinosa-Garcia and Langenheim 1990), and grapevine (Mostert et al. 2000, Rawnsley et al. 2004).

Often, the terms latent infection and endophyte can be misleading. Latent infection refers to the period of time in which the host is infected but symptoms are not apparent (Agrios 1988), and implies that a parasitic relationship is dormant or quiescent and will, in time, become an active parasite (Verhoeff 1974). While this is seldom disputed, a gray area encompassing both terms of latent infection and endophyte exists and results in misunderstanding. The term endophyte often carries the assumption that the microbe-host relationship is mutualistic (Wilson 1995, Saikkonen et al. 1998, Saikkonen et al. 2004). However, most reported definitions of the term maintain that the definition does not imply a role for the endophyte, mutualistic or otherwise, and simply implies that an organism lives inside the plant host (Petrini 1991, Schulz 1998). Thus pathogens with latent phases as well as opportunistic pathogens that will result in

symptoms on stressed plants are included in the term endophyte, and the relationship of an endophyte and the host plant can be antagonistic or mutualistic. The references listed above that report species of *Phomopsis* as an endophyte all refer to an endophyte as either a non-pathogenic fungus, or a fungus that results in disease infrequently.

According to the predominant definitions as well as conventional wisdom, the isolates of *P. vaccinii* and *Phomopsis* sp. that resulted in low incidence of disease can be referred to as endophytes. More specifically, some *Phomopsis* sp. isolates (ATCC 56789, FAU 445, and FAU 453) and *P. vaccinii* isolate DF 5044 did not result in disease development in any trial. Considering the fact that these fungi were also isolated from symptomless tissue indicates that these fungi may be non-pathogenic inhabitants of cranberry. However, there is no information to speculate if these isolates result in a benefit to the plant, such as herbivore determent or production of antimicrobials. *Phomopsis* sp. isolates DF 5025, DF 5036, DF 5041 and *P. vaccinii* isolate CBS 160.32 resulted in a low percent, less than 35%, of replicate plants developing symptoms. That these fungi were also isolated from both diseased and symptomless tissues indicates that, while these isolates will most often be non-pathogenic, these isolates can result in the development of symptoms.

It can be concluded that *Discula* sp. isolates are not stem pathogens of 'Bluejay' blueberry plants. Also, as discussed in the proof-of-pathogenicity trials (Chapter 2), it was previously concluded that *Discula* sp. was not a pathogen of cranberry stems.

Susceptibility of Cultivars 'Early Black' and 'Stevens'

In the field, it is observed that 'Early Black' cranberry vines appear more susceptible to upright dieback than 'Stevens' vines (Caruso, personal communication).

Since trials were conducted at different times, it is difficult to compare the susceptibility of 'Early Black' and 'Stevens' cranberry cultivars. However, with the consideration of the various trials in this study along with the experiments discussed earlier, some observations can be made. More isolate treatments resulted in dieback symptoms on inoculated 'Early Black' tissue-cultured cranberry plants than 'Stevens' tissue-cultured cranberry plants. These results are consistent with the results from the proof-of-pathogenicity trials using tissue cultured plants in which the results indicated that 'Early Black' plants were more sensitive to infection (Chapter 2). This conclusion was based on the observation that symptom development and tissue death occurred generally more rapidly after inoculation in the 'Early Black' plants than the 'Stevens' plants. In contrast, in the proof-of-pathogenicity trial using cranberry rooted cuttings, 'Stevens' plants developed symptoms of tissue death more rapidly after inoculation than did 'Early Black' plants (Chapter 2). However, these trials were conducted in the greenhouse at different times – the 'Stevens' trial initiated in August 2002 and the 'Early Black' trial initiated in October 2002. Although the 'Early Black' plants were maintained under lights (12 h of light per day), these plants were not growing as vigorously as the 'Stevens' plants, and this difference likely explains the differences observed in the symptom development between the two cultivars in these experiments. Symptom development was also observed to be different between the two infection-court trials conducted with 'Stevens' cranberry rooted cuttings in which trials initiated in the fall differed from trials initiated in the spring (Chapter 2). Although *P. vaccinii* inoculation resulted in greater amounts of tissue death in the fall trial, some tested infection courts, such as non-wounded plants, showed symptoms in the spring trial

when they did not show dieback symptoms in the fall trial. These results indicated that while, in some cases, the amount of tissue death was greater when infection occurred late in the growing season, cranberry plant growth was more sensitive to infection during early spring. In order to clearly discern the differences in susceptibility of various cultivars, it is necessary to inoculate plants of the same source and growth stage concurrently.

Comparison of Cranberry Trials – 'Early Black' and 'Stevens' Tissue Culture, and 'Early Black' Cranberry Rooted Cuttings

Inoculation with the various isolates caused differing symptom development on the two cultivars of tissue-cultured cranberry plants and the cranberry rooted cuttings. Particularly noticeable were the differences between the tissue-cultured plants and the rooted cuttings. For example, *P. vaccinii* isolates DF 5022 and ATCC 18451 both resulted in 88.9% of inoculated rooted cuttings showing dieback symptoms, while these isolates both only produced symptoms on 12.5% of inoculated 'Early Black' tissue-cultured plants and no symptoms in 'Stevens' tissue-cultured cranberry plants. Also, *Phomopsis* sp. isolate DF 5041 and *P. vaccinii* isolate CBS 160.32 only resulted in symptom development on rooted cuttings and did not result in symptoms on any tissue-cultured plants. In previous trials, tissue-cultured plants and rooted cuttings responded similarly to inoculation with *P. vaccinii*, isolate 98023 (Chapter 2), while *Discula* sp. isolate 93009C resulted in symptom development on a small percent of tissue-cultured plants, but not on any rooted cuttings.

Due to the susceptibility of tissue-cultured plants and the ideal environment for fungal growth in the tissue-culture vial, it was expected that tissue-cultured cranberry

plants would be most sensitive to fungal infection. The tissue-cultured cranberry plants were not wounded and the agar inoculum was simply placed on the plant, while the rooted cuttings were wound-inoculated and the inoculum was sealed with sterile moistened Kimwipes® and Parafilm®. The differences in symptom development between the tissue-culture trials and the rooted-cutting trial in this study are likely explained by the fact that cranberry rooted cuttings were wound-inoculated and the tissue-cultured plants were not wounded in their inoculation procedure. In the proof-of-pathogenicity studies previously discussed (Chapter 2), wound-inoculated tissue-cultured plants developed symptoms more quickly than non-wounded plants. It is possible that the differences between the rooted cuttings and the tissue-culture trials would not have been observed if the tissue-cultured plants could have been monitored for a longer length of time.

Table 4.1. Fungi used in the pathogenicity tests conducted on rooted cuttings of blueberry plants. All fungi were isolated from stem tissue of blueberry, *Vaccinium corymbosum*, and cranberry, *Vaccinium macrocarpon.*

Isolate	Fungus	Collector	Host	Origin	Year
control	---	N/A	N/A	N/A	N/A
93009C [a]	*Discula* sp.	F.L. Caruso	cranberry	MA	1993
93011B [a]	*Discula* sp.	F.L. Caruso	cranberry	MA	1993
93012B [a]	*Discula* sp.	F.L. Caruso	cranberry	MA	1993
93020A	*Phomopsis vaccinii*	F.L. Caruso	blueberry	MA	1993
98020	*Discula* sp.	F.L. Caruso	cranberry	MA	1998
98021	*Phomopsis vaccinii*	F.L. Caruso	cranberry	MA	1998
98023	*Phomopsis vaccinii*	F.L. Caruso	cranberry	MA	1998

[a] These isolates were previously identified as *Phomopsis* sp., but were recently determined to be morphologically similar to species of *Discula*, though not similar based on sequence analysis of rDNA. (Castlebury, personal communication). Until a more suitable genus name is defined and for the purpose of this dissertation, these isolates will be referred to as *Discula* sp.

Table 4.2. Fungi used in the pathogenicity tests conducted on tissue-cultured cranberry plants and rooted cuttings of cranberry plants. All fungi were isolated from stem tissue of blueberry, *Vaccinium corymbosum*, and cranberry, *Vaccinium macrocarpon,* except where otherwise noted.

Isolate	Fungus	Collector	Host	Origin	Year
control	N/A	N/A	N/A	N/A	N/A
93020A	*Phomopsis vaccinii*	F.L. Caruso	blueberry	MA	1993
98023	*Phomopsis vaccinii*	F.L. Caruso	cranberry	MA	1998
ATCC 18451	*Phomopsis vaccinii*	R.J. Friend	cranberry	WI	1968
ATCC 56789 [a]	*Phomopsis* sp.	D.A. Glawe	cranberry	WI	1983
CBS 160.32	*Phomopsis vaccinii*	C.L. Shear	cranberry	MA	1932
DF 5022	*Phomopsis vaccinii*	D.F. Farr	blueberry	NC	1999
DF 5025	*Phomopsis* sp.	D.F. Farr	blueberry	NC	1999
DF 5036	*Phomopsis* sp.	D.F. Farr	blueberry	NC	1999
DF 5041	*Phomopsis* sp.	D.F. Farr	blueberry	NC	1999
DF 5044	*Phomopsis vaccinii*	D.F. Farr	blueberry (fruit)	NC	1999
FAU 445 [a]	*Phomopsis* sp.	F.L. Caruso	cranberry	MA	1992
FAU 453	*Phomopsis* sp.	J.E. Gilbert	blueberry	OR	1987

[a] Based on analysis rDNA, these isolates belong to a well-supported groups of isolates from a wide range of hosts that includes isolates identified as *Diaporthe eres* from *Acer* and *Corylus* (Castlebury 2005, personal communication).

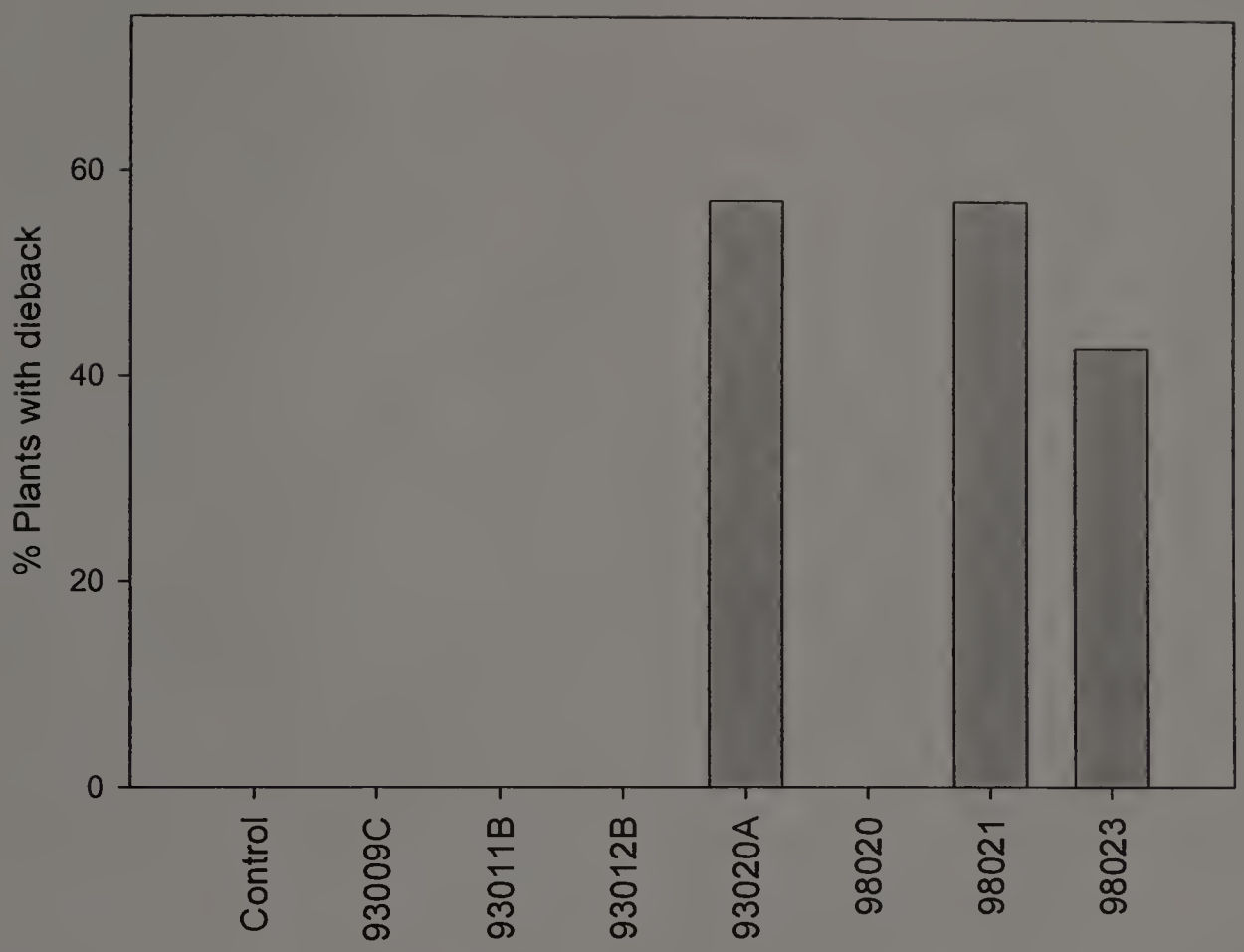


Figure 4.1. Average percent of 'Bluejay' blueberry plants showing dieback symptoms after inoculation with various isolates of *P. vaccinii* and *Discula* sp. in pathogenicity trials conducted in 2001 and 2002 (N=14).

Table 4.3. Summary of the percent of plants with dieback symptoms after inoculation with different *Phomopsis* sp. or *P. vaccinii* isolates in three different trials – blueberry rooted microshoots ('Bluejay') (N=14), 'Early Black' tissue-cultured cranberry plants (EB – TC) (N=8), 'Stevens' tissue-cultured cranberry plants (ST – TC) (N=8), and 'Early Black' cranberry rooted cuttings (EB – RC) (N=9).

Isolate	Fungus	Host	Tissue	Origin	% plants with dieback			
					Blueberry	EB – TC	ST – TC	EB –RC
control	N/A	N/A	N/A	N/A	0	0	0	0
93009C	*Discula* sp.	cranberry	stem	MA	0	N/A	N/A	N/A
93011B	*Discula* sp.	cranberry	stem	MA	0	N/A	N/A	N/A
93012B	*Discula* sp.	cranberry	stem	MA	0	N/A	N/A	N/A
98020	*Discula* sp.	cranberry	stem	MA	0	N/A	N/A	N/A
93020A	*Phomopsis vaccinii*	blueberry	stem	MA	57.1	62.5	62.5	100.0
DF 5022	*Phomopsis vaccinii*	blueberry	stem	NC	N/A	12.5	0	88.9
DF 5044	*Phomopsis vaccinii*	blueberry	fruit	NC	N/A	0	0	0
98021	*Phomopsis vaccinii*	cranberry	stem	MA	64.3	N/A	N/A	N/A
98023	*Phomopsis vaccinii*	cranberry	stem	MA	35.7	50.0	62.5	88.9
ATCC 18451	*Phomopsis vaccinii*	cranberry	stem	WI	N/A	12.5	0	88.9
CBS 160.32	*Phomopsis vaccinii*	cranberry	stem	MA	N/A	0	0	33.3
ATCC 56789	*Phomopsis sp.*	cranberry	stem	WI	N/A	0	0	0
FAU 445	*Phomopsis sp.*	cranberry	stem	MA	N/A	0	0	0
DF 5025	*Phomopsis sp.*	blueberry	stem	NC	N/A	28.6	0	11.1
DF 5036	*Phomopsis sp.*	blueberry	stem	NC	N/A	28.6	12.5	33.3
DF 5041	*Phomopsis* sp.	blueberry	stem	NC	N/A	0	0	33.3
FAU 453	*Phomopsis sp.*	blueberry	stem	OR	N/A	0	0	0

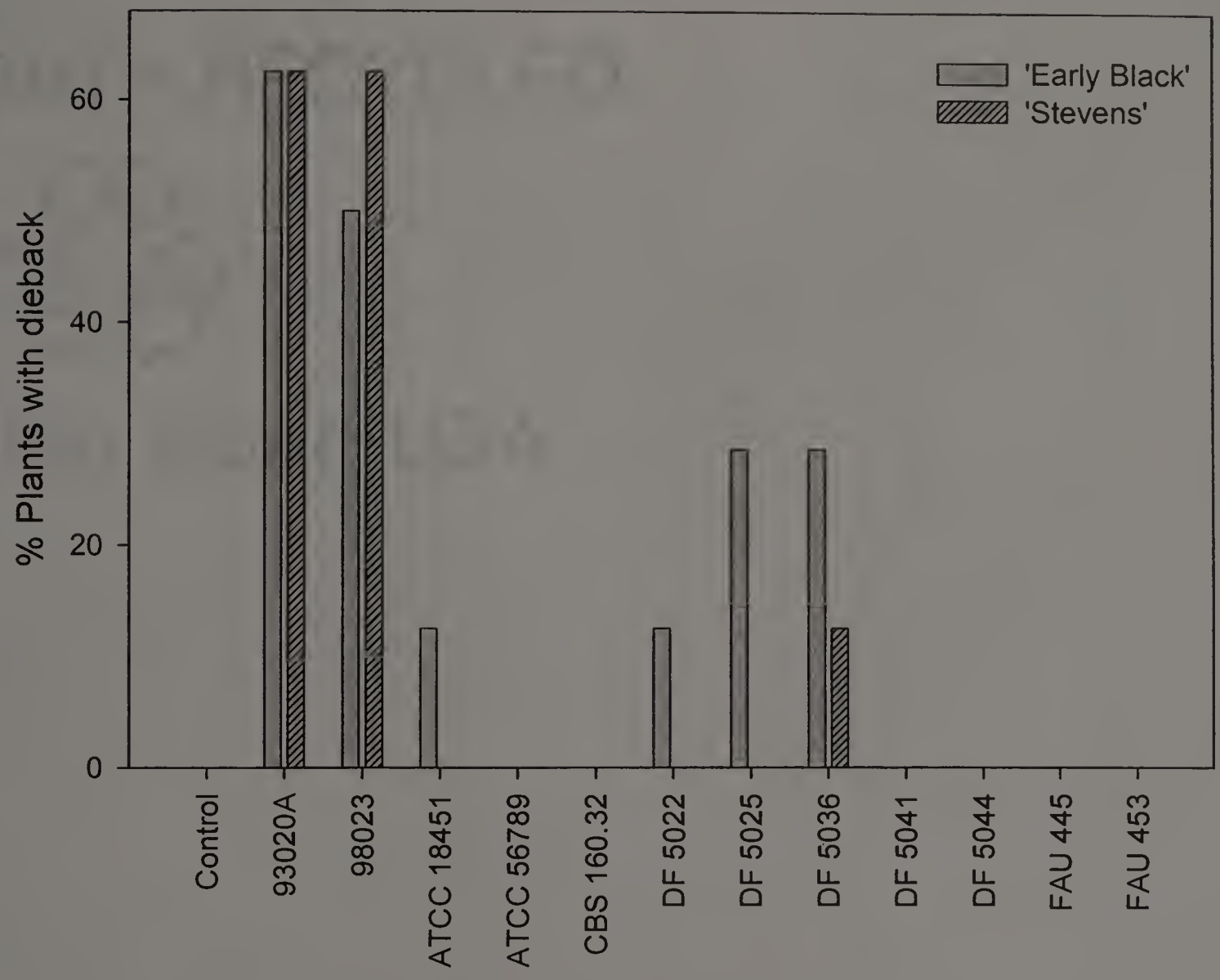


Figure 4.2. Percent of 'Early Black' and 'Stevens' plants showing dieback symptoms 50 days after inoculation with various isolates of *P. vaccinii* and *Phomopsis* sp. (N=8).

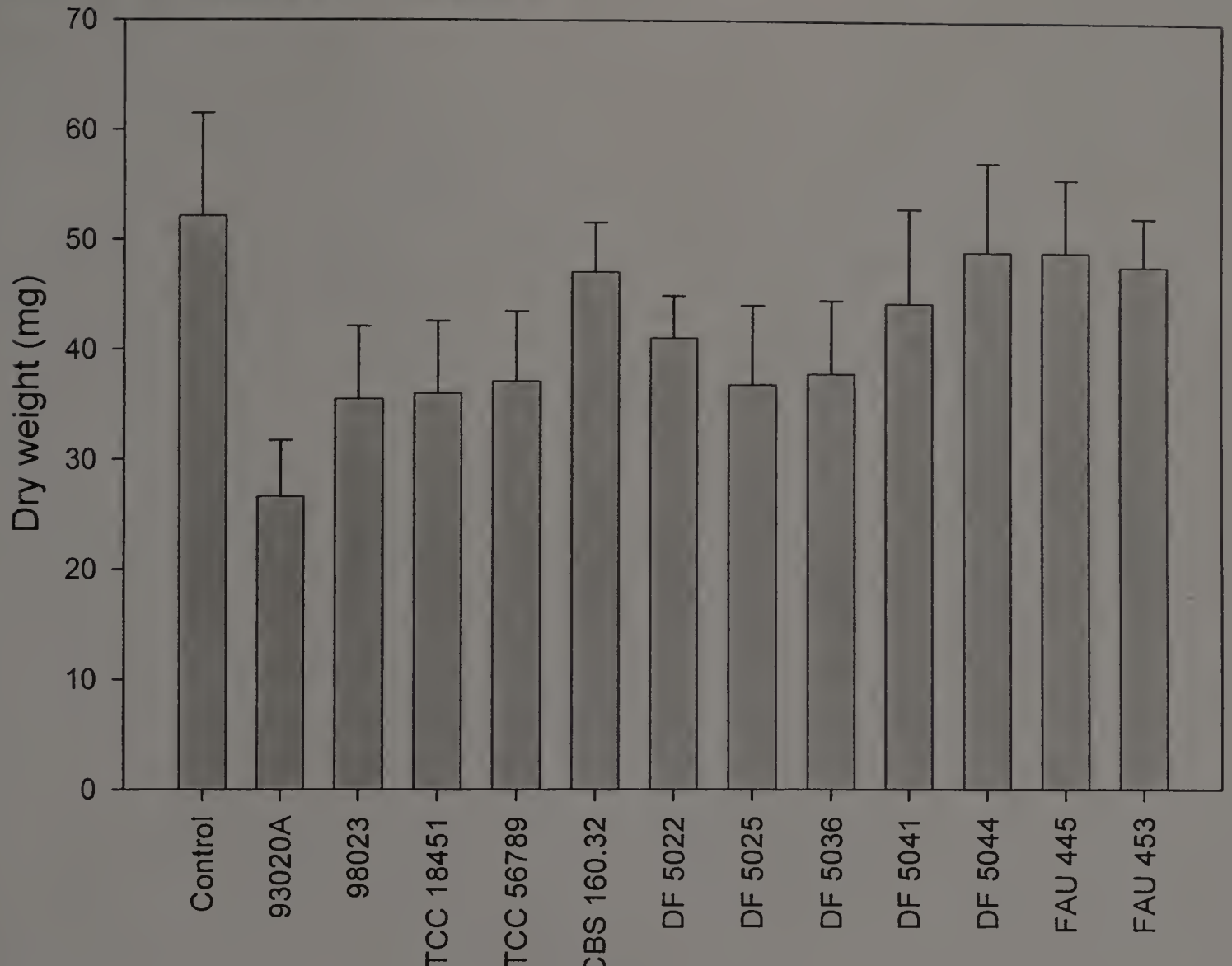


Figure 4.3. Dry weight of 'Early Black' plants at the termination of a pathogenicity trial in which tissue-cultured cranberry plants were inoculated with various isolates of *P. vaccinii* and *Phomopsis* sp. (N=8). Vertical bars represent standard error of the means.

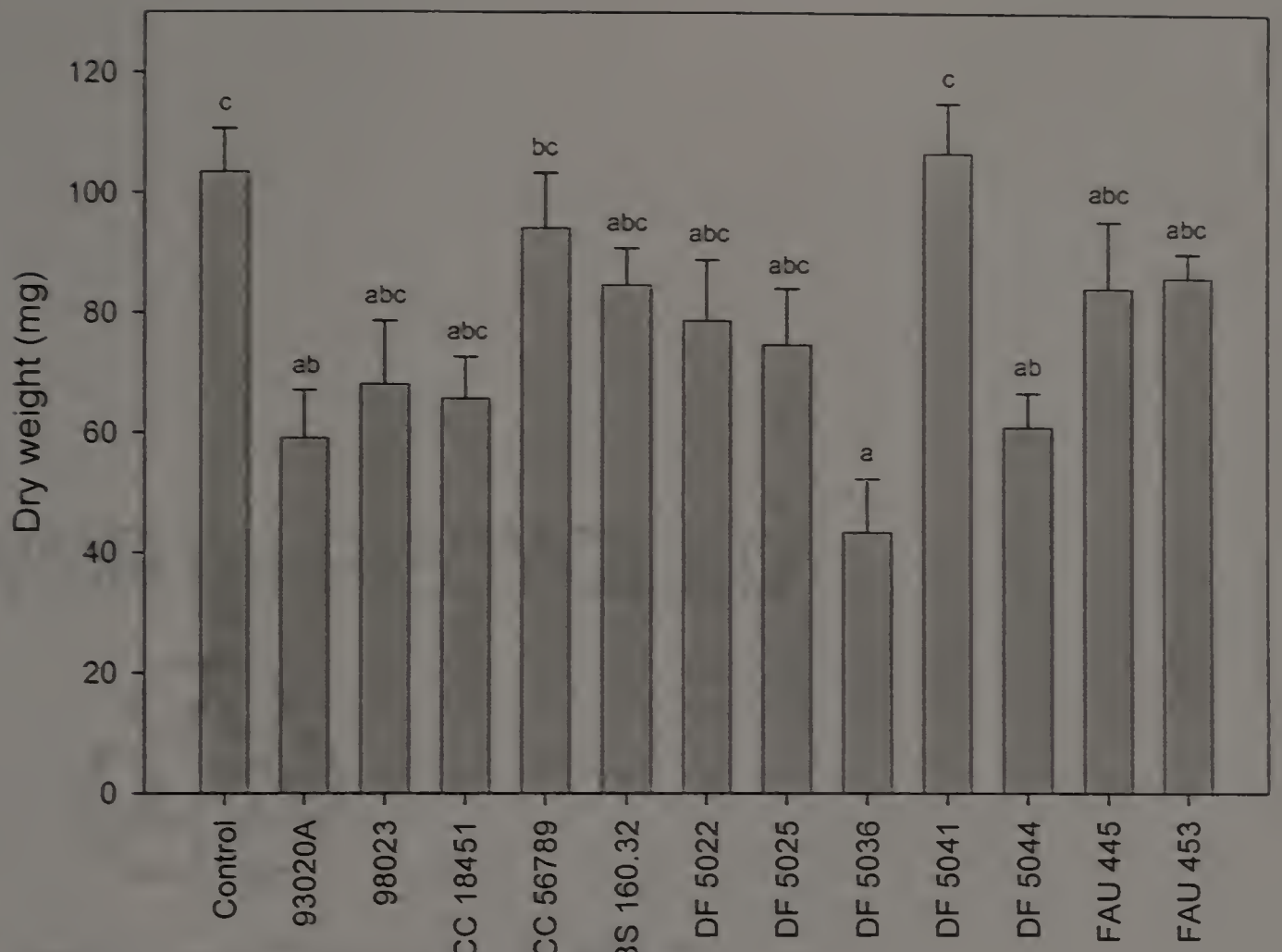


Figure 4.4. Dry weight of ' 'Stevens' plants at the termination of a pathogenicity trial in which tissue-cultured cranberry plants were inoculated with various isolates of *P. vaccinii* and *Phomopsis* sp. (N=8). Vertical bars represent standard error of the means. Means with similar letters are not significantly different according to Kramer-adjusted Tukey's HSD (p = 0.05).

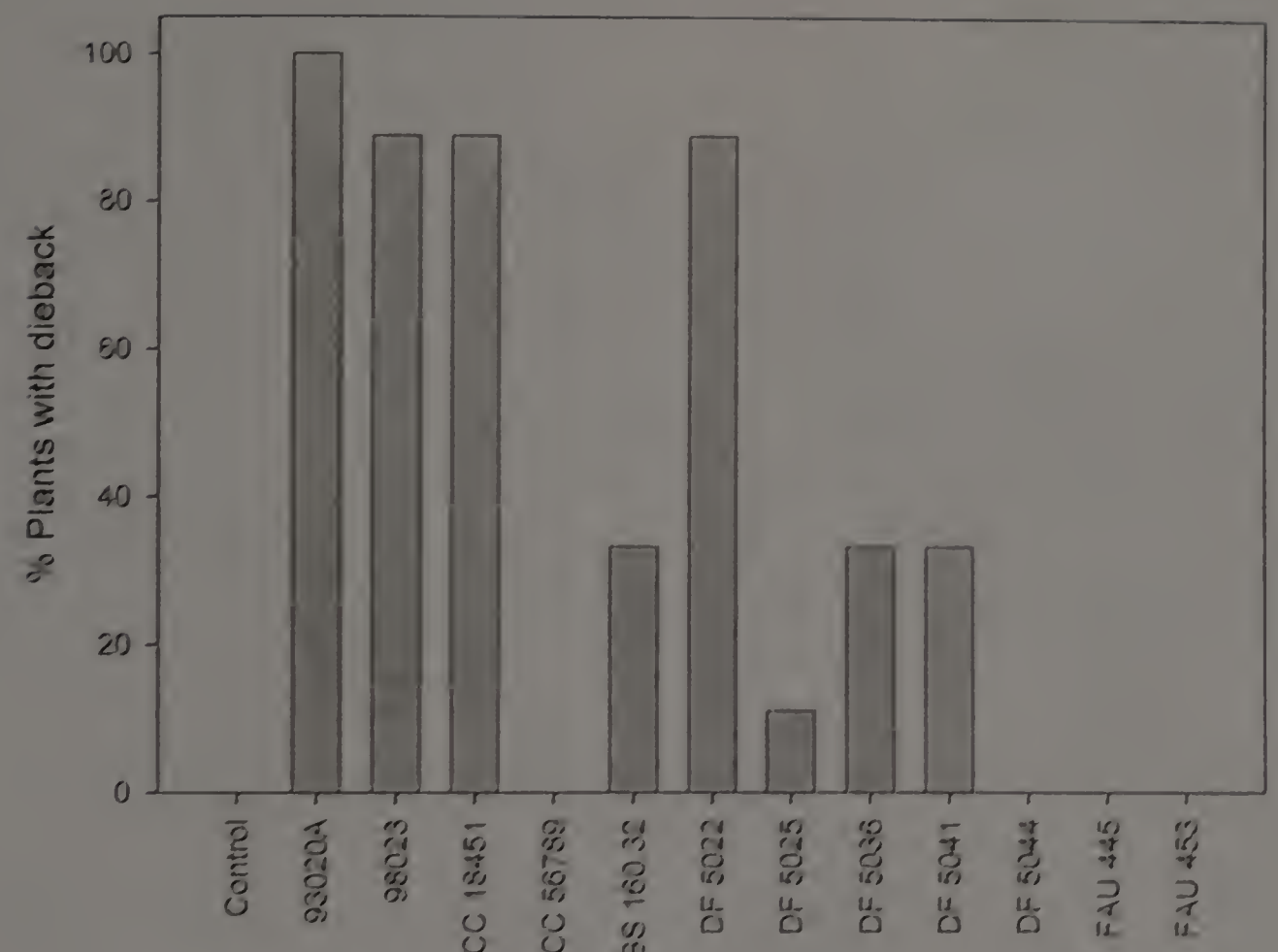


Figure 4.5. Percent of cranberry rooted cuttings ('Early Black') showing dieback symptoms 60 days after inoculation with various isolates of *P. vaccinii* and *Phomopsis* sp. (N=9).

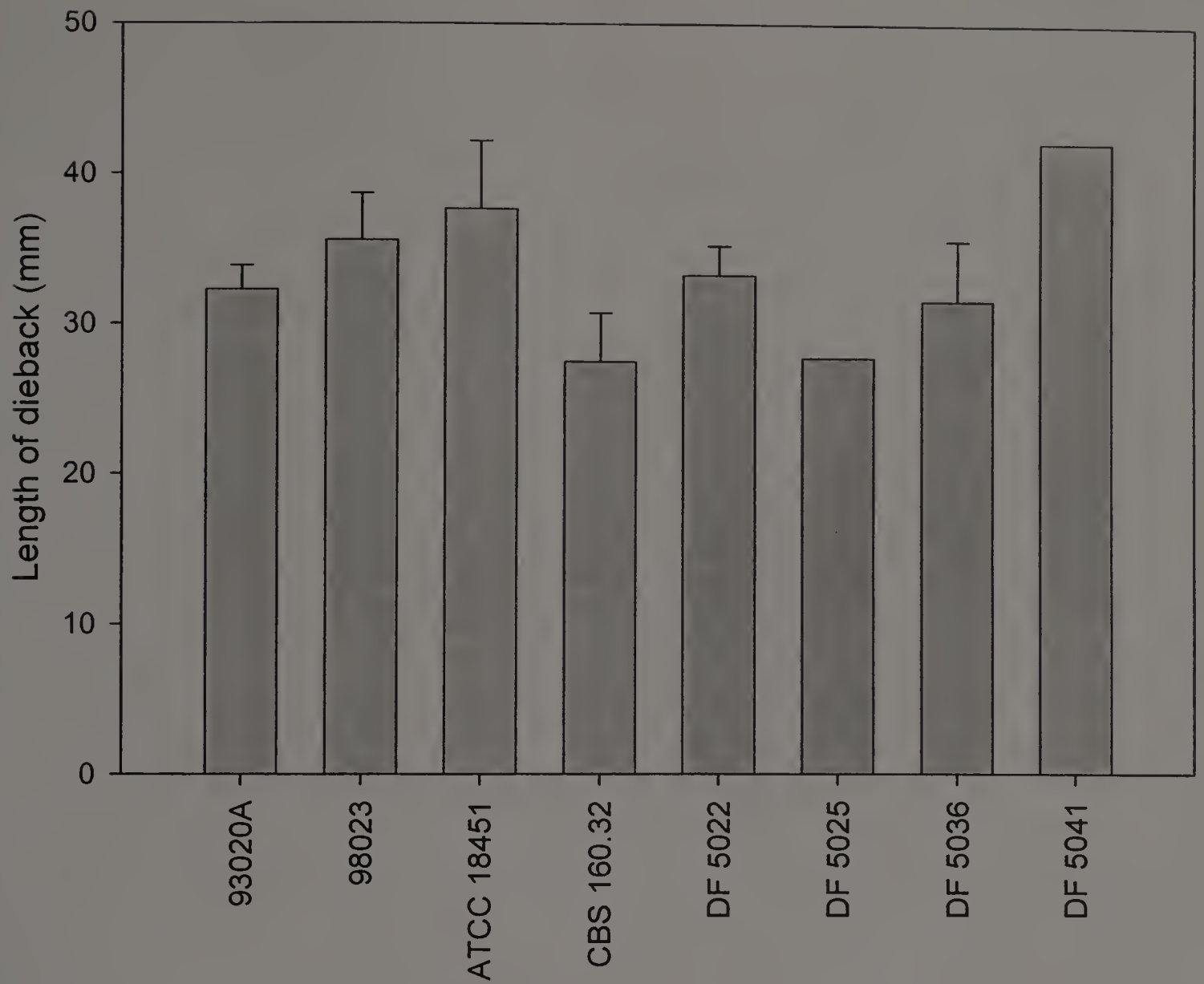


Figure 4.6. Length of dieback of cranberry rooted cuttings ('Early Black') exhibiting symptoms 60 days after inoculation with various isolates of *P. vaccinii* and *Phomopsis* sp. (98020A: N=9, 98023: N=8, ATCC 18451: N=7, CBS 160.32: N=3, DF 5022: N=8, DF 5025: N=1, DF 5036: N=3, DF 5041: N=3). Vertical bars represent standard error of the means.

CHAPTER 5

HISTOLOGY OF TISSUE-CULTURED CRANBERRY PLANTS INFECTED WITH *PHOMOPSIS VACCINII*

Introduction

As discussed in Chapters 2 and Chapter 4, *Phomopsis vaccinii* and other *Phomopsis* sp. were determined to be causal agents of upright dieback disease of cranberry, *Vaccinium macrocarpon*. Discolored internal tissues have been observed on uprights affected with upright dieback, suggesting the possibility of a vascular nature of upright dieback disease (Dana et al. 1967, Caruso and Ramsdell 1995). Additionally, *P. vaccinii* infection resulting in twig dieback of blueberry, *Vaccinium corymbosum*, has been determined to be systemic. The fungus infected leaves, flowers, or stem tissue and progressed into and throughout the stem tissue, colonizing the cortical tissues and eventually the vascular tissues (Milholland 1982, Daykin and Milholland 1990). The objective of this study was to investigate which types of cranberry tissues *P. vaccinii* infects and colonizes.

Materials and Methods

Infected tissue-cultured cranberry plants were chosen for microscopic examination for various reasons. Since tissue-cultured plants are grown in sterile culture, it is assured that any fungus observed microscopically is the introduced fungus. In addition, tissue-cultured plants are more succulent than cranberry field plants and are therefore less problematic to section.

Fresh cuttings of tissue-cultured plants, cv. Stevens, were established. The origin and maintenance of the tissue-cultured plants were as described in earlier chapters (Chapter 2). These tissue-cultured plants were inoculated with *P. vaccinii* isolate 98023, which was also used for the proof-of-pathogenicity trials, infection-court trials, and isolate-test trials (Chapters 1, 2, and 3). Plants were inoculated with plugs of agar and mycelium with a non-wound technique, as described earlier (Chapter 2).

Eight replicate plants were inoculated in mid-September 2002. To ensure that the appropriate stages of infection were available when the samples were processed, four additional replicate plants were inoculated 14, 21, and 36 days after the first inoculation. Samples of plants showing symptoms were taken 48 days after the first set of inoculations. Samples of symptomatic stem and leaf tissue near the point of inoculation were selected for microscopic observation.

Samples were fixed overnight at room temperature in 2% paraformaldehyde, 2.5% glutaraldehyde in 0.025M Na-phosphate buffer (pH 7.0). Samples were triple washed in 0.050M Na-phosphate buffer (pH 7.0) for 20 min each, then washed and rinsed in distilled water. After washing and rinsing, the samples were dehydrated in a series of ethanol (30, 50, 70, 80, 95, 100, 100, and 100%) on ice and transferred to acetone (100%).

Samples were infiltrated with firm Spurr's resin by 3:1, 1:1, and 1:3 acetone:Spurr's resin steps (2 h each), uncapped in a fume hood draft for 2 h, transferred to fresh Spurr's resin, and rotated overnight. Finally, samples were transferred to fresh Spurr's resin on rotator for 4 h, then embedded in additional Spurr's resin and polymerized at 70C for 48 h.

Blocks were cut from the resin and mounted for sectioning. Sections, approximately 1 µm, were cut on a Reichert Ultracut microtome with glass knives, and gently dried onto glass slides. The sections were then stained with 0.05% Toluidine Blue-O in 1% sodium borate, rinsed, and dried. Coverglasses were mounted with immersion oil. Samples were observed at 20 to 100X under white light using a Nikon E-600 microscope.

Results

Hyphae of *P. vaccinii* were observed throughout the dead leaf tissue as well as on the surface of the leaf (Figure 5.1). All cell types appeared colonized, including the xylem, and parenchyma and phloem cells were collapsed.

P. vaccinii was also observed in the stem sections of infected tissue-culture plants (Figure 5.2). Infection was observed to be confined to a small area of vascular tissue in the cross sections examined. A host response was observed in the infected stem tissue (Figures 5.3 and 5.4). Cells in this area appeared hyperplastic, smaller, and more numerous, than the cells in the healthy portions of the stem section. Hypertrophic cells and the presence of phenolic compounds were observed outside the area of infection, and thickened and suberized cell walls were observed between healthy and infected areas.

Discussion

Of the leaf sections examined, *P. vaccinii* was observed on the leaf surface and throughout all leaf tissues. The leaf tissue that was observed was dead tissue, and it was not surprising to find that *P. vaccinii* invaded all or most of the leaf tissues. Since the

sections observed were at such a late stage in the infection process, information about fungal penetration and the early stages of leaf infection was not observed.

The stem tissues that were observed were predominantly healthy, but showed a small necrotic area on the stem at the point of inoculation – a typical symptom of early infection of tissue-culture plants. In the stem tissues, *P. vaccinii* was also observed in the vascular tissue. The vascular nature of *P. vaccinii* infection of cranberry explains the reported association of increased incidence of upright dieback during periods of heat stress (Friend and Boone 1968, Caruso and Ramsdell 1995).

It is proposed that systemic infection of *P. vaccinii* occurs through stem wounds, as seen in the infection-court studies presented earlier (Chapter 3), or systemic infection occurs as a foliar infection which advances to the stem tissue. Systemic *P. vaccinii* infection of highbush blueberry occurs via infection of flower tissues, leaf margins, or stem wounds (Milholland 1982). In a histological study, blueberry flower buds or opened flower clusters were inoculated with a conidial suspension of *P. vaccinii* (Daykin and Milholland 1990). It was reported that succulent flower parts were first invaded by the fungus, after which the fungus moved through the intra-and inter-cellular spaces of cortex of the floral axis and pedicel into the intra- and intercellular spaces of the stem cortex. While *P. vaccinii* was often observed in the vascular tissues and the pith, these tissues were not invaded until the cortex was completely colonized.

A different infection process occurs in *Phomopsis helianthi* infection of sunflower (*Helianthus annuus*). Muntañola-Cvetković et al. (1989, 1991) reported that stem infection by *P. helianthi* was initiated by a foliar infection that spread to the stem

via the vascular tissues. In this case, after the vascular tissue was invaded and colonized, the fungus spread to invade other tissues.

Since only a few sections of *P. vaccinii*-infected cranberry leaf and stem tissue were observed, the exact infection process can not be determined. It is expected that the infection process of *P. vaccinii* of cranberry stems is similar to the infection process observed in the closely related plant blueberry.

A host response was observed in the infected tissue-cultured cranberry plants. Hyperplastic cells were observed in the infected region of the stem. Hypertrophic cells, suberized cells, and phenolic compounds were observed in the cells exterior to the infection. While these host responses were observed, fungal growth was still present in the sections viewed. Further investigation is needed in order to determine at what point the host defenses would be successful.

A host response was also observed in *P. vaccinii*-infected blueberry plants. Daykin and Milholland (1990) reported hypertrophied cells below infection sites on ovaries and hyperplastic cells below infection sites on floral axes. In addition, numerous tyloses were observed in advance of fungal growth and suberized cells developed between infected and healthy portions of the infected blueberry stems. Regardless, these responses did not prevent the fungus from further growth and the authors suggested the possibility that a physiological response was finally responsible for limiting fungal growth.

For naturally infected cranberry plants, symptoms develop only on current-year tissue. On some *P. vaccinii*-inoculated plants, for example non-wound-inoculated plants, it was observed that only a small amount of the current-year growth showed

dieback symptoms and the plant developed a new shoot below the infected tissue (Chapter 2). Based on the symptom development of naturally infected plants as well as inoculated plants. it is assumed that some host response is ultimately successful at limiting the spread of *P. vaccinii* throughout the stem tissue.

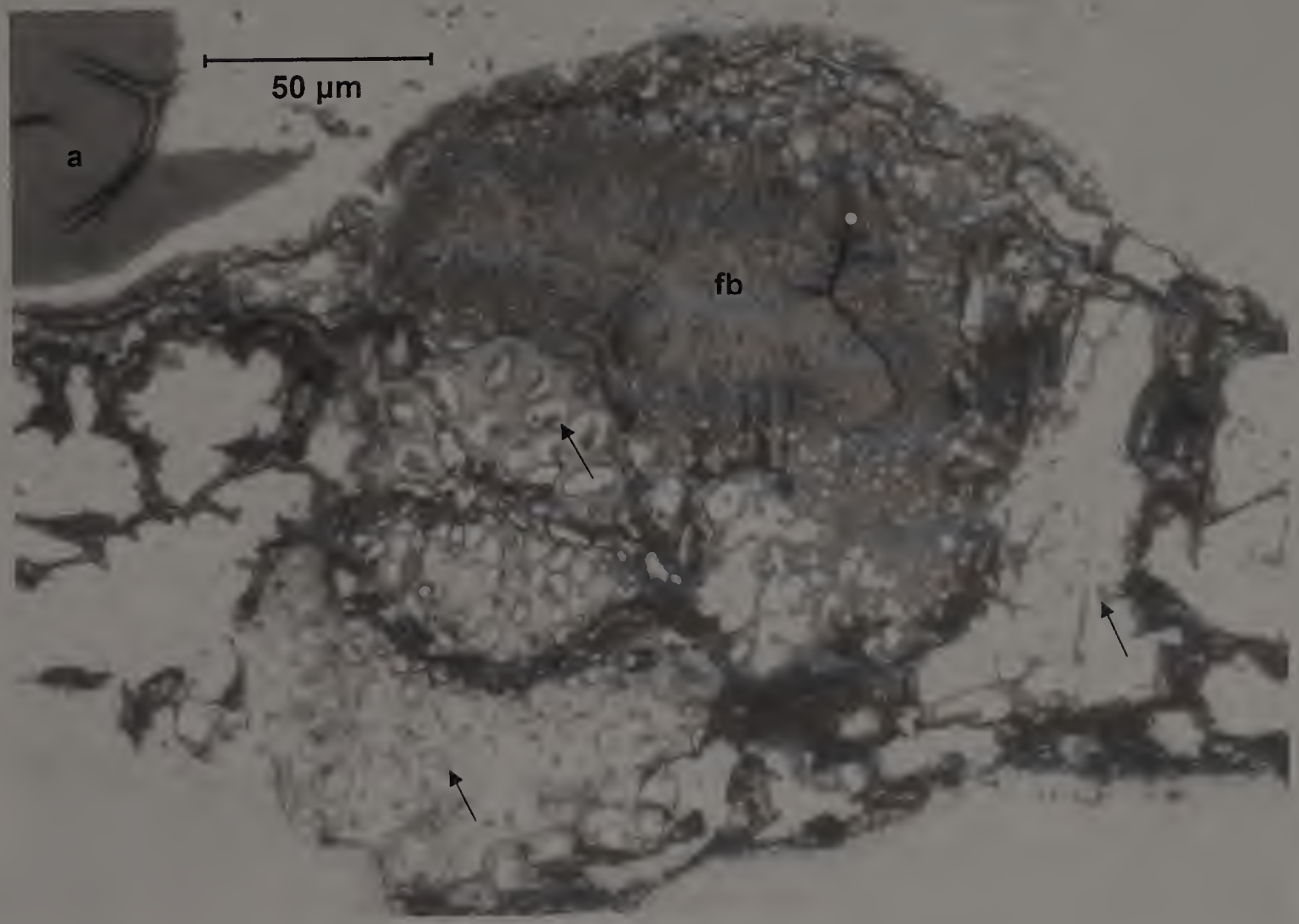


Figure 5.1. Cross-section of a leaf of a *P. vaccinii*-infected tissue-cultured cranberry plant showing tissues thoroughly colonized by the fungus. The agar-mycelium plug used as inoculum (a) can also be seen in this section. Hyphae of *P. vaccinii* can be observed on leaf surface, and in all tissues (examples are indicated by arrows); parenchyma and phloem cells appear collapsed. A developing fruiting body (fb) can be observed under the epidermis.

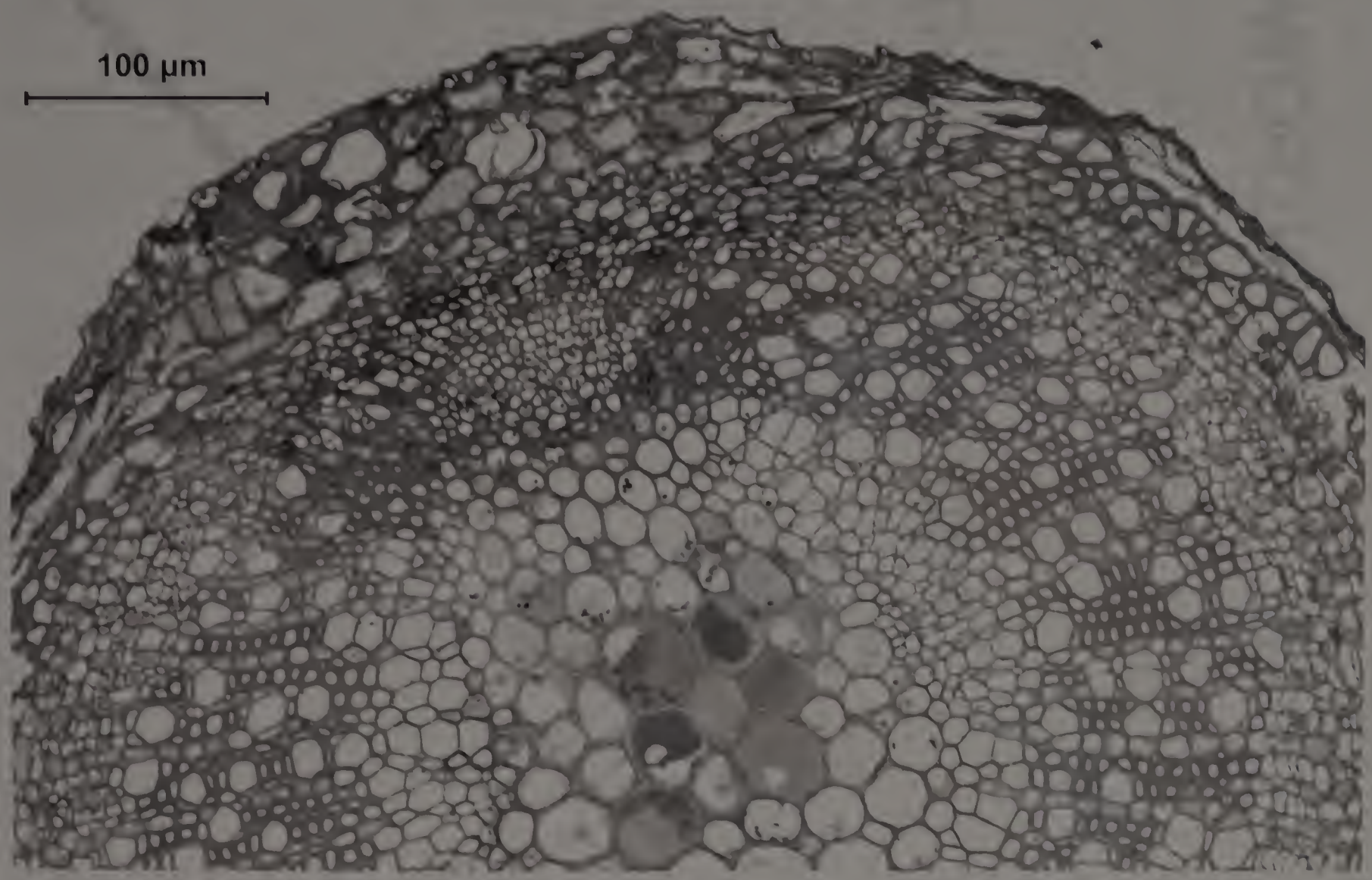


Figure 5.2. Cross-section of a *P. vaccinii*-infected tissue-cultured cranberry stem showing a small region of fungal colonization and host responses. Hyperplastic cells can be observed in infected region, hypertrophic and phenolic compounds to the outside of the infection, and thickened, suberized cell walls in cells bordering the infected region.

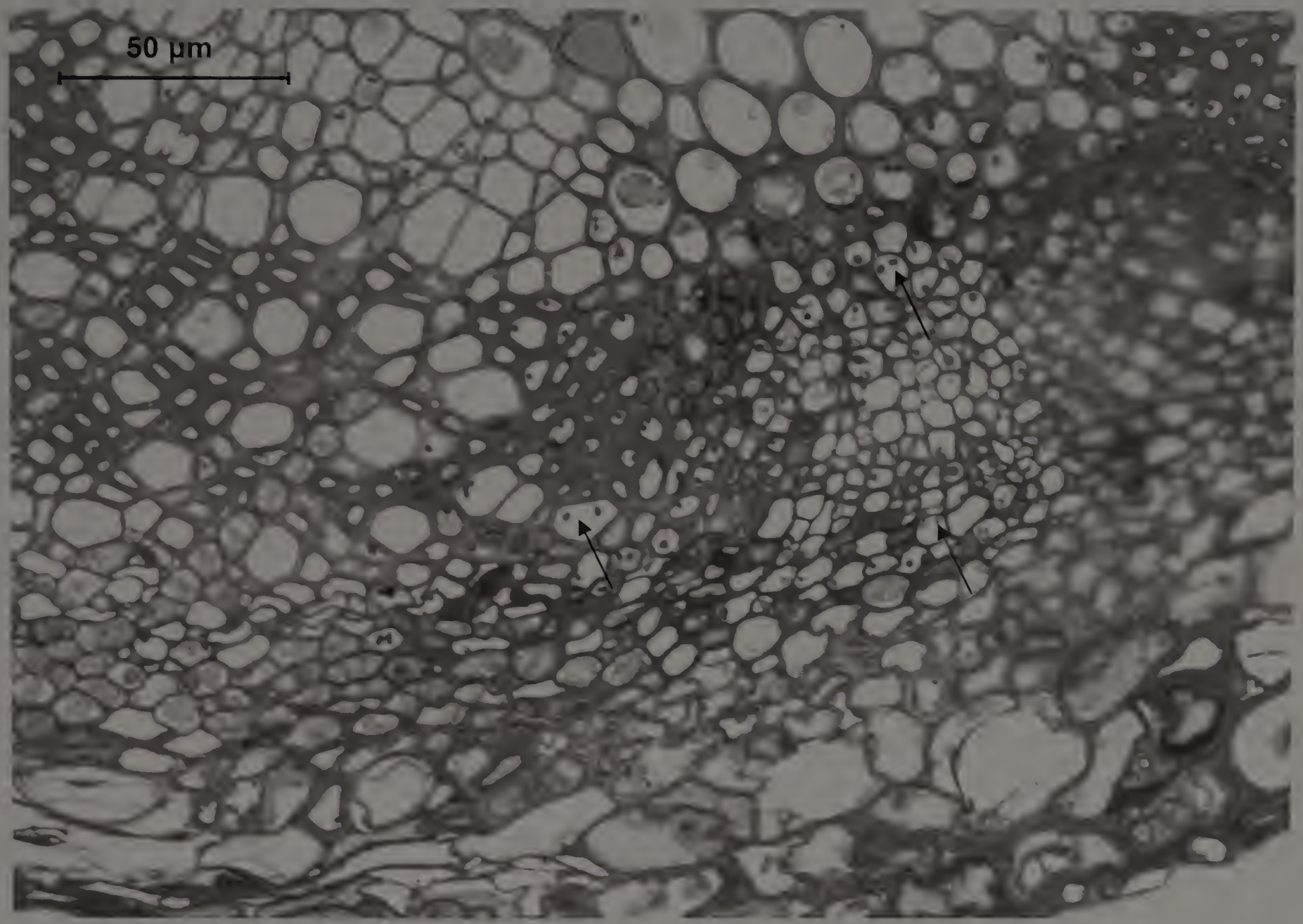


Figure 5.3. Additional view of a cross-section of a *P. vaccinii*-infected tissue-cultured cranberry stem showing fungal colonization of vascular tissue (examples indicated by arrows).

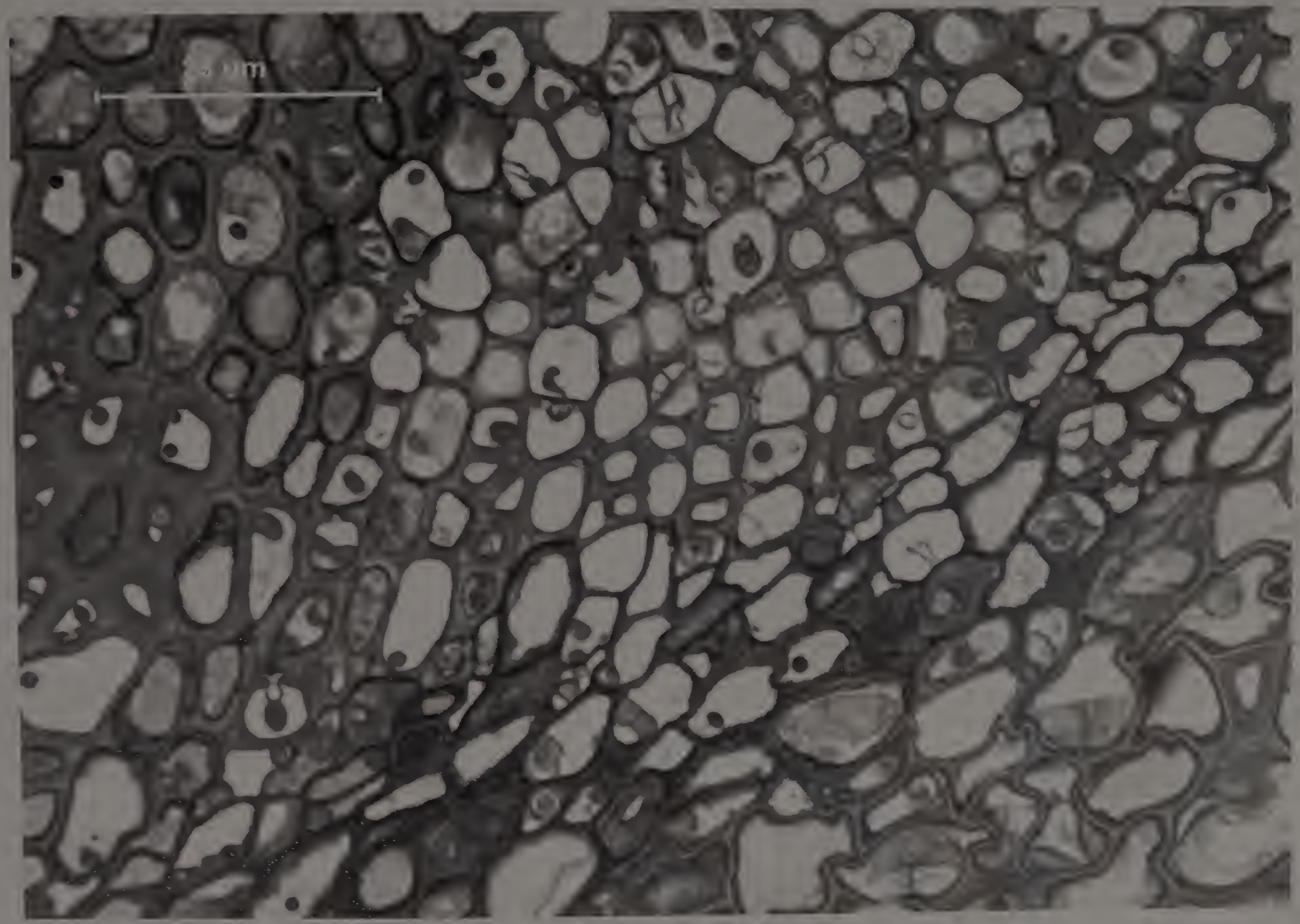


Figure 5.4. Additional view of a cross-section of a *P. vaccinii*-infected tissue-cultured cranberry stem showing fungal colonization of vascular tissue .

CHAPTER 6

DISSERTATION SUMMARY

Dissertation Summary

P. vaccinii and *Phomopsis* sp. result in the development of upright dieback symptoms on inoculated cranberry tissue-cultured plants and rooted cuttings. One *P. vaccinii* isolate and a few *Phomopsis* sp. isolates resulted in disease development on no inoculated plants, or only a low percent of plants. *Discula* sp. resulted in disease development on only a low percent of inoculated tissue-cultured plants and did not result in symptom development or any response on inoculated cranberry rooted cuttings or rooted microshoots of blueberry. Since the isolates of *P. vaccinii*, *Phomopsis* sp. and *Discula* sp. that resulted in no or low incidence of disease were regularly recovered from symptomless tissue, these isolates appear to be non-parasitic, or rarely parasitic, endophytes of cranberry plants.

Cranberry plants appear most susceptible to infection in the spring when fresh growth is present, though the uprights and runners are susceptible throughout the season if wounded. At all points in the growing season, wounded plants, are more susceptible to infection and often develop more extensive tissue death than non-wounded plants. The research presented in this dissertation supports the current recommended control measures of managing the cranberry bed to avoid drought stress and rank vine growth, and an early spring application of a protective fungicide. However, the efficacy of these fungicide applications is questioned. Additional control measures were suggested:

avoiding plant wounds by protecting the plants from freezing injury and limiting foot traffic, pruning, or other mechanical damage in the spring when vines are particularly susceptible and inoculum is likely to be high.

The results of this dissertation indicate that *P. vaccinii* is a vascular pathogen and colonizes the stem tissue via a stem wound, or the fungus infects succulent leaves and progresses from the leaf tissue into the stem tissue, eventually colonizing vascular tissue.

Future Research

While the research presented here provided some answers to the role of *Phomopsis* in upright dieback disease of cranberry, numerous questions remain. Listed below are additional questions whose answers would be a useful addition to the knowledge base of *P. vaccinii* and *Phomopsis* sp. infection, upright dieback disease, and the management of the disease.

- What are the effects of upright dieback on yield?
- What are the infection periods, the periods of high inoculum, and the sources of inoculum of *P. vaccinii* and *Phomopsis* sp.?
- What is the efficacy of early spring protective fungicide sprays, and what is the optimal timing for disease management?
- What is the role of environmental effects, such as leaf wetness and heat stress, and role of physiological stress, such as low carbohydrate availability, in infection and disease development?

- What is the link between upright dieback and fruit rot (viscid rot)?
- Which fungi also frequently isolated from diseased uprights can incite upright dieback, if any?
- What is the association of the apparently non-pathogenic isolates of *P. vaccinii* and *Phomopsis* sp. and the cranberry plant?

APPENDIX

PRELIMINARY SURVEY OF FUNGI FOUND ON CRANBERRY BEDS IN MASSACHUSETTS

Introduction

Several fungi are frequently cultured from uprights with symptoms of upright dieback: *Aureobasidium pullulans*, *Colletotrichum acutatum*, *Colletotrichum gloeosporiodes*, *Fusicoccum putrefaciens*, *Gloeosporium minus*, *Pestalotia vaccinii*, and *Phomopsis vaccinii* (Friend and Boone 1968, Caruso unpublished data).

Friend and Boone (1968) isolated various fungi from diseased and apparently healthy cranberry stems including *Phomopsis vaccinii*, *F. putrefaciens*, and species of *Alternaria*, *Epicoccum*, and *Fusarium*. Only *P. vaccinii* and *Fusarium* spp. were isolated more frequently from diseased stems than from symptomless stems, and since *Fusarium* spp. was only occasionally isolated from diseased and healthy stems, it was concluded that *P. vaccinii* was most likely the causal agent of upright dieback. In an earlier study conducted by the same authors (Friend and Boone 1968), it was determined that *P. vaccinii* was isolated more frequently from beds with dieback than from beds apparently free of the disease.

Preliminary data indicate that most of the above listed fungi can be isolated from apparently healthy tissue as well as from diseased tissue (Catlin and Caruso 2001). Since many of the fungi listed above have been isolated from uprights exhibiting dieback symptoms and apparently healthy uprights, it is possible that these fungi are either latent pathogens or are normally associated with upright tissue. It is expected

that latent pathogens would be recovered at a higher frequency from beds with a history of upright dieback than from beds apparently free from the disease. The objectives of this introductory study were to determine which fungi are currently associated with apparently healthy uprights and uprights exhibiting dieback symptoms in Massachusetts, to compare the recovery of fungi from upright tissue from cranberry beds with and without a history of upright dieback, and to determine which fungi might be causal agents of upright dieback.

Materials and Methods

Pairs of cranberry beds, one with a history of upright dieback and one without a history of upright dieback, were identified through University of Massachusetts Cranberry Station disease clinic records and communication with growers. The pairs of beds were proximal, of the same cultivar and with as similar as possible management practices (Table A.1).

Samples were collected three times from each cranberry bed – collections occurred in mid-July, mid-August and mid-September. For each sample collection, 50 healthy uprights were collected from all beds, and 30 to 50 diseased uprights were collected only from beds with a history of upright dieback. Stem pieces, approximately 2 cm, were taken from the sampled uprights for plating. Of the healthy stem pieces, two stem sections of were plated – the current-year growth and the 1-yr-old growth. Current-year growth refers to the fresh tissue of new flush of growth and 1-yr-old growth refers to the woody tissue of the past-year's growth. For the diseased uprights, the margin of diseased and healthy tissue was plated. The stem pieces were surface disinfested by soaking for 10 s in 70% ethanol (ETOH) followed by 2 min in 0.5%

sodium hypochlorite (NaOCl) plus Tween80® solution. After surface disinfestation, the stem pieces were plated onto acidified corn meal agar (ACMA), and incubated at room temperature until the fungi present could be identified.

For each sample, percent recovery for each fungus isolated from the various tissue samples were determined. The various tissue samples were: symptomless current-year growth sampled from beds without a history of dieback (current-year growth/healthy beds), symptomless 1-yr-old growth sampled from beds without a history of dieback (1-yr-old growth/healthy beds), symptomless current-year growth sampled from beds with a history of dieback (current-year growth/dieback beds), symptomless 1-yr-old growth sampled from beds with a history of dieback (1-yr-old growth/dieback beds), and diseased tissue sampled from beds with a history of upright dieback (diseased tissue). For each fungus, the percent recovery data were subjected to analysis of variation (ANOVA) (SAS V 9.1, SAS Institute), and when necessary, data were transformed prior to analysis to better meet ANOVA assumptions. When significant effects were determined, means were separated using Kramer-adjusted Tukey's honestly significant difference (HSD).

At the end of the season, 10 random 6-inch diameter samples of uprights were excised from each bed and disease incidences were recorded. The data for each pair of beds were subjected to ANOVA. When necessary, data were transformed to better meet ANOVA assumptions.

Results

The following fungi were routinely isolated from the various tissue samples: *Alternaria* sp., *Aspergillus* sp., *Aureobasidium pullulans*, *Botrytis* sp., *Cladosporium*

sp., *Coleophoma empetri* (Rostr.) Petr., *Colletotrichum acutatum*, *Colletotrichum gloeosporiodes*, *Epicoccum* sp., *Fusicoccum putrefaciens*, *Gloeosporium minus*, *Helminthosporium* sp., *Paecillomyces* sp., *Penicillium* spp., *Pestalotia vaccinii* (*Pe. vaccinii*), *Phoma* sp., *Phomopsis vaccinii* (*P. vaccinii*), *Phyllosticta* spp., *Physalospora vaccinii* (Shear) Arx & E. Muller (*Phy. vaccinii*), and *Sphearopsis vaccinii* W.B. Cooke & C.G. Shaw.

Fungi isolated in 2001 from symptomless current-year growth sampled from beds with and without a history of upright are presented in Figures A.1 and A.2, respectively. Fungi isolated from symptomless 1-yr-old growth sampled from beds with and without a history of upright dieback are presented in Figures A.3 and A.4, respectively. Fungi isolated from diseased uprights are presented in Figure A.5.

After the post-season evaluation of disease incidence, it was determined that only three of the seven pairs of beds had the expected levels of upright dieback – the beds with a supposed history of dieback had a significantly greater incidence of upright dieback than beds that were supposedly free from the disease (Figure A.6). The only pairs that for which the percent upright dieback was significantly different between the beds were Pair 3, (Carver 3A and Carver 3B) ($p = 0.0476$), Pair 4 (E. Wareham A and E. Wareham B) ($p = 0.006$), and Pair 7 (Wareham 2A and Wareham 2B) ($p = 0.0329$). Since the relationships between the incidences of upright dieback were only significantly different between the beds in three of the seven pairs, only these three pairs of beds will be further discussed for the 2001 study.

In 2002, samples were collected only from the three pairs of beds that had significantly different incidences of upright dieback in 2001. Fungi isolated from

symptomless current-year growth sampled from beds with and without a history of upright dieback are shown in Figures A.7 and A.8, respectively. Fungi isolated from symptomless 1-yr-old growth sampled from beds with and without a history of upright dieback are presented in Figures A.9 and A.10, respectively. Fungi isolated from diseased uprights are presented in Figure A.11. The percent of uprights affected with dieback in 2002 are shown in Figure A.12. Of the three pairs sampled in 2002, only beds of two pairs had significantly different incidences of dieback, Pair 3 (Carver 3A and Carver 3B) ($p = 0.0328$), and Pair 4 (E. Wareham A and E. Wareham B) ($p = 0.0360$). Only these two pairs will be further discussed for the 2002 study.

The percent recovery of fungi from symptomless tissue and diseased tissue was compared for the samples taken in 2001 (Figure A.13) and 2002 (Figure A.14). Additionally, the percent recovery from symptomless tissue sampled from beds with and without a history of upright dieback was compared. The percent recovery of fungi isolated from symptomless current-year growth tissue sampled from beds with and without a history of upright dieback is presented in Figure A.15 (2001) and Figure A.16 (2002), and the percent recovery of fungi from symptomless 1-yr-old growth tissue sampled from beds with and without a history of upright dieback is presented in Figure A.17 (2001) and Figure A.18 (2002).

Numerous fungi were frequently isolated from diseased uprights. In 2001 *P. vaccinii* was recovered from an average of greater than 20% of plated diseased tissue (Figure A.13). *Alternaria* sp., *A. pullulans*, *F. putrefaciens*, *Cladosporium* sp., and *G. minus* were all recovered from an average of 10 to 20% of plated diseased tissue, and all other fungi were recovered from less than 10% of plated samples of diseased tissue. In

2002, *Cladosporium* sp., *F. putrefaciens* and *P. vaccinii* were recovered from greater than 20% of plated diseased tissue samples of all three collections (Figure A.14). *Alternaria* sp., *A. pullulans*, and *Pe. vaccinii* were recovered from an average of 10 to 20% of plated diseased tissue samples, and all other fungi were recovered from less than 10% of plated diseased tissue samples.

Of the fungi isolated from more than 10% of the plated diseased tissue samples in 2001, *A. pullulans*, *Cladosporium* sp., *G. minus*, and *P. vaccinii* were recovered more frequently from diseased uprights than from symptomless uprights (Figure A.13). Of these four fungi, only *Cladosporium* sp. was recovered more frequently from symptomless current-year growth sampled from beds with dieback than from beds without dieback (Figure A.15), and only *A. pullulans* and *Cladosporium* sp. were recovered more frequently from symptomless 1-yr-old growth sampled from beds with dieback compared to beds without dieback (Figure A.17).

Of the fungi isolated from more than 10% of the plated diseased tissue samples in 2002, *A. pullulans*, *Cladosporium* sp., *F. putrefaciens*, *Pe. vaccinii* and *P. vaccinii* were recovered more frequently from diseased uprights than from symptomless uprights (Figure A.14). Of these five fungi, only *Cladosporium* sp. and *P. vaccinii* were recovered more frequently from symptomless current-year growth sampled from beds with dieback compared to beds without dieback (Figure A.16), and only *Pe. vaccinii* and *P. vaccinii* were recovered more frequently from symptomless 1-yr-old growth sampled from beds with dieback compared to beds without dieback (Figure A.18).

A few fungi were selected for further investigation and statistical analysis. The fungi reported to be frequently isolated from stems exhibiting dieback, discussed

previously (Chapter 1), *A. pullulans*, *C. acutatum*, *C. gloeosporiodes*, *F. putrefaciens*, *G. minus*, *Pe. vaccinii*, and *P. vaccinii* were selected. In addition, *Cladosporium* sp. was selected since this fungus was isolated frequently from diseased uprights (Figures A.13 and A.14) and was isolated more frequently from symptomless tissue sampled from beds with a history of upright dieback than from symptomless tissue sampled from beds without a history of the disease (Figures A.16 to A.19).

Few differences in the recovery of fungi from different tissue types were significant (Tables A.2 and A.3). For both years of the survey, many of the fungi had significant differences in the fungal recovery between the collection times, or a significant interaction between tissue samples and collection time. However, only the fungi with significant differences in the recovery from the various tissue samples will be discussed in more detail.

In 2001, the percent recovery between the different tissue samples were only significantly different for *P. vaccinii*. *P. vaccinii* was recovered from more diseased tissue samples than from symptomless samples (Figure A.19). However, only the percent recovery from diseased tissue and symptomless 1-yr-old growth were significantly different. Also, the percent recovery of *P. vaccinii* was significantly different between the collection times. Collection 3, taken in September, was significantly different from Collections 1 and 2, taken in July and August, respectively (Figure A.20).

In 2002, only *F. putrefaciens* and *Pe. vaccinii* had percent recoveries that were significantly different between the different tissue samples. In 2002, *F. putrefaciens* was recovered with the highest frequency from symptomless 1-yr-old growth collected

from beds with dieback, symptomless 1-yr-old growth collected from beds without dieback, and from diseased tissue (Figure A.21). However, percent recovery from diseased tissue was not significantly different from any samples of symptomless tissue. *Pe. vaccinii* was recovered with the highest frequency from diseased tissue (Figure A.22). However, only the percent recovery from diseased tissue and percent recovery from symptomless 1-yr-old growth tissue sampled from beds with upright dieback was significantly different.

Discussion

In these studies, numerous fungi were frequently isolated from diseased uprights including *Alternaria* sp., *A. pullulans*, *Cladosporium* sp., *F. putrefaciens*, *G. minus*, *Pe. vaccinii*, and *P. vaccinii*. Few differences between the percent recovery of these fungi from the various tissue samples were significant. However, it can be observed that only *A. pullulans*, *Cladosporium* sp., and *P. vaccinii* were recovered more frequently from diseased uprights than from symptomless uprights in both years of the survey. These three fungi were also typically isolated more frequently from symptomless tissue from beds with dieback than from beds without dieback. The percent recovery of these fungi from symptomless tissue from beds with dieback and without dieback were typically not consistent between the two years of the survey or significantly different. *Cladosporium* sp. was recovered more frequently from symptomless current-year growth and 1-yr-old growth sampled from beds with dieback than from beds without dieback in 2001 and 2002. In 2001 and 2002, *A. pullulans* was recovered more frequently from 1-yr-old growth sampled from beds with dieback compared to beds without dieback. In 2002, *P. vaccinii* was recovered more frequently from symptomless

current-year growth and symptomless 1-yr-old growth sampled from beds with dieback compared to beds without dieback.

The results of these studies indicate that many of the fungi reported to be frequently isolated from uprights with dieback symptoms and considered to be possible causal agents are not likely to have a role in upright dieback. *C. acutatum* and *C. gloeosporiodes* were not frequently recovered from diseased tissue (<10% of the plated uprights), and should not be considered causal agents. *G. minus* was recovered from a higher percent of diseased uprights than from symptomless uprights, but was only recovered from 13% of plated diseased tissue in 2001 and 8% of plated diseased tissue in 2001. Therefore, *G. minus* is also an unlikely pathogen due to its low recovery. *F. putrefaciens* was inconsistently isolated from diseased tissue during the 2 years the survey was conducted. *F. putrefaciens* was only recovered from 13% of plated diseased tissue samples and 16% of plated symptomless tissue samples in 2001. In contrast, in 2002 the fungus was recovered from 41% of plated diseased samples and 31% of symptomless tissue samples. Unless it is determined that *F. putrefaciens* is consistently isolated from diseased tissue, this fungus should not be considered a possible causal agent of upright dieback.

The only fungi that were consistently isolated from a high percentage of diseased uprights and also isolated more frequently from diseased uprights than from symptomless uprights were *A. pullulans*, *Cladosporium* sp., and *P. vaccinii*. Typically, these three fungi were isolated more frequently from symptomless tissue sampled from beds with dieback than from beds without dieback. The results from this study indicate that *A. pullulans* and *Cladosporium* sp. may be considered for future research as fungi

that may be involved in dieback of cranberry. However, *P. vaccinii* remains the most likely pathogen based on the data from this survey, previous surveys (Friend and Boone 1968 and Caruso and Ramsdell 1995), and the involvement of *P. vaccinii* in twig dieback and canker diseases of highbush blueberry (Wilcox 1939, Weingartner and Klos 1975, and Parker and Ramsdell 1977).

Table A.1. Management and cultivar information of the pairs of cranberry beds surveyed for incidence of fungi.

Pair	History of dieback	No history of dieback	Cultivar	Management notes
1	Carver 1A	Carver 1B	Howes	Carver 1A: early fungicide [a] 2001
2	Carver 2A	Carver 2B	Early Black	Carver 2A: late water [b] 2001 Carver 2B: late water 2001
3	Carver 3A	Carver 3B	Early Black	Carver 3A: late water 2001 Carver 3B: crop destruct [c] 2001
4	East Wareham A	East Wareham B	Howes	East Wareham A: sand [d] 2002
5	Hanson A	Hanson B	Stevens	Hanson B: late water 2001
6	Wareham 1A	Wareham 1B	Howes	
7	Wareham 2A	Wareham 2B	Early Black	

[a] Early fungicide: an early spring fungicide applied at bud break to control upright dieback (Sandler 1998).
[b] Late water: a cultural practice for the control of various pests in which a flood is applied to the cranberry bed for approximately one month during some springs (DeMoranville et al. 2005).
[c] Crop destruct: a flood applied to a cranberry bed during bloom when the bed will not be used for berry production (DeMoranville 2001).
[d] Sand: a cultural practice completed every 3 to 8 years in which a 2 to 5 cm layer of sand is applied to the bog to encourage rooting of buried runners and uprights and reducing the populations of some insect and inoculum of some diseases (DeMoranville and Sandler 2000).

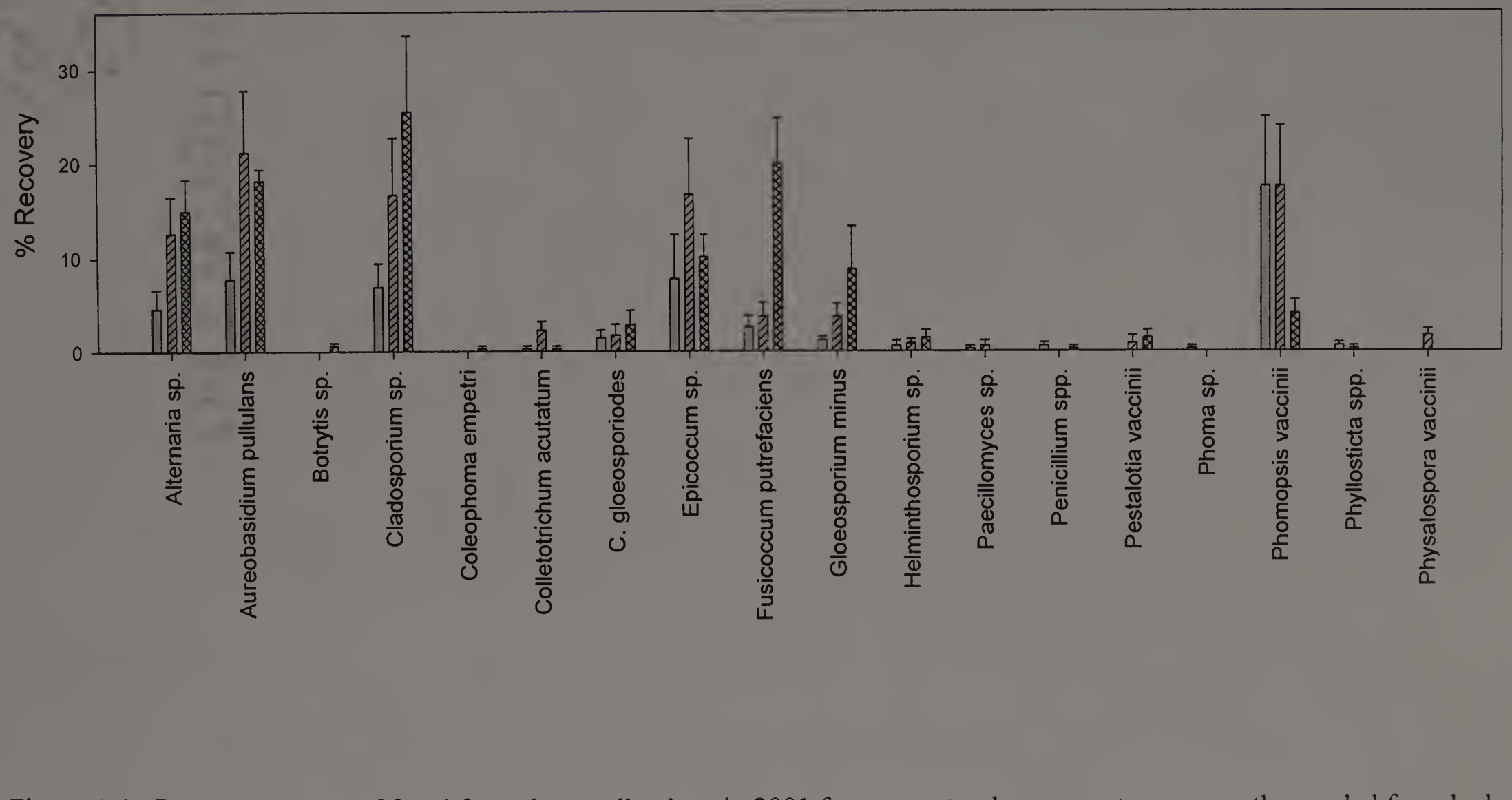


Figure A.1. Percent recovery of fungi from three collections in 2001 from symptomless current-year growth sampled from beds with a history of upright dieback (N=7). Vertical bars represent standard error of the means.

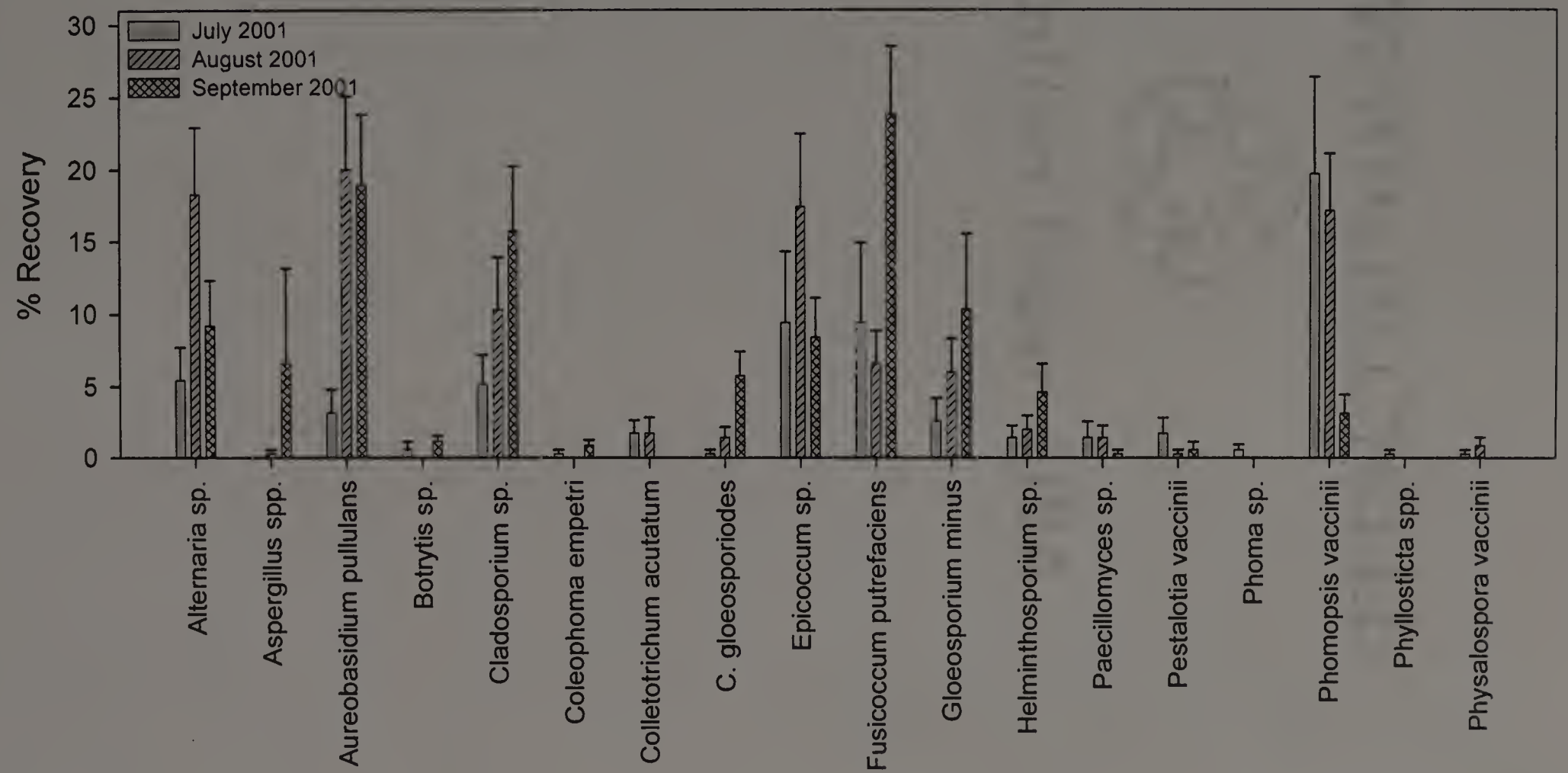


Figure A.2. Percent recovery of fungi from three collections in 2001 from symptomless current-year growth sampled from beds without a history of upright dieback (N=7). Vertical bars represent standard error of the means.

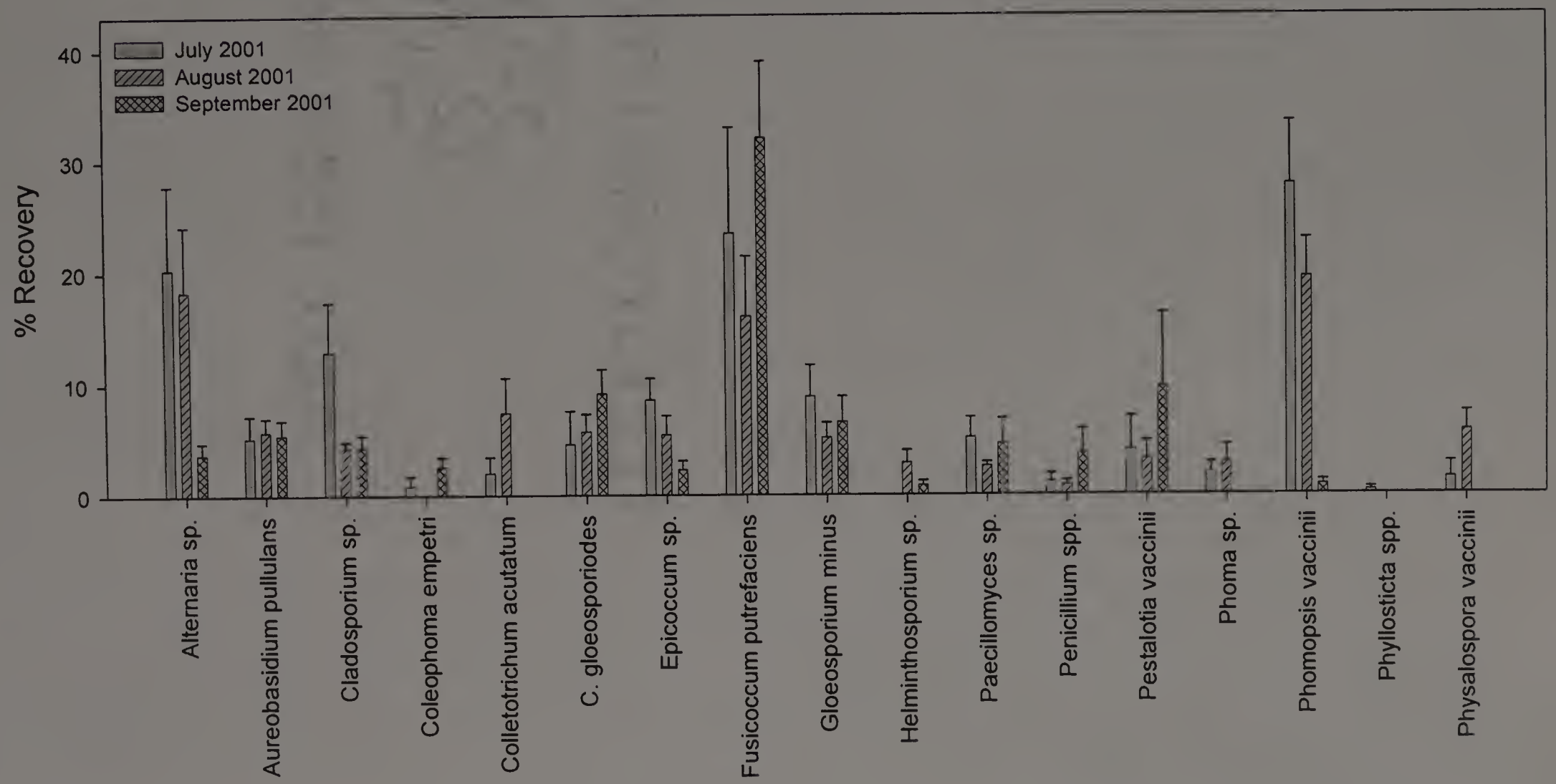


Figure A.3. Percent recovery of fungi from three collections in 2001 from symptomless one-year-old growth sampled from beds with a history of upright dieback (N=7). Vertical bars represent standard error of the means.

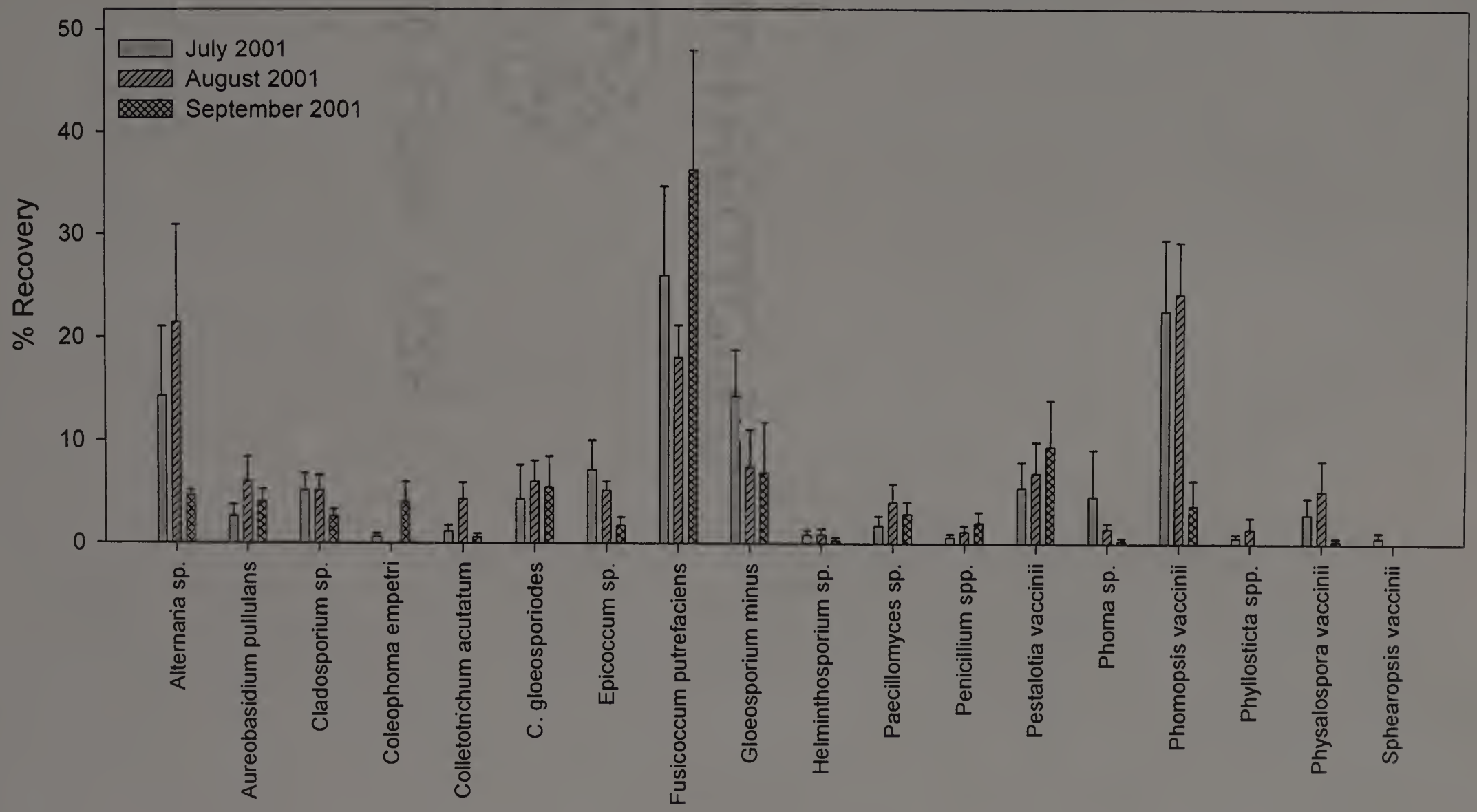


Figure A.4. Percent recovery of fungi from three collections in 2001 from symptomless one-year-old growth sampled from beds without a history of upright dieback (N=7). Vertical bars represent standard error of the means.

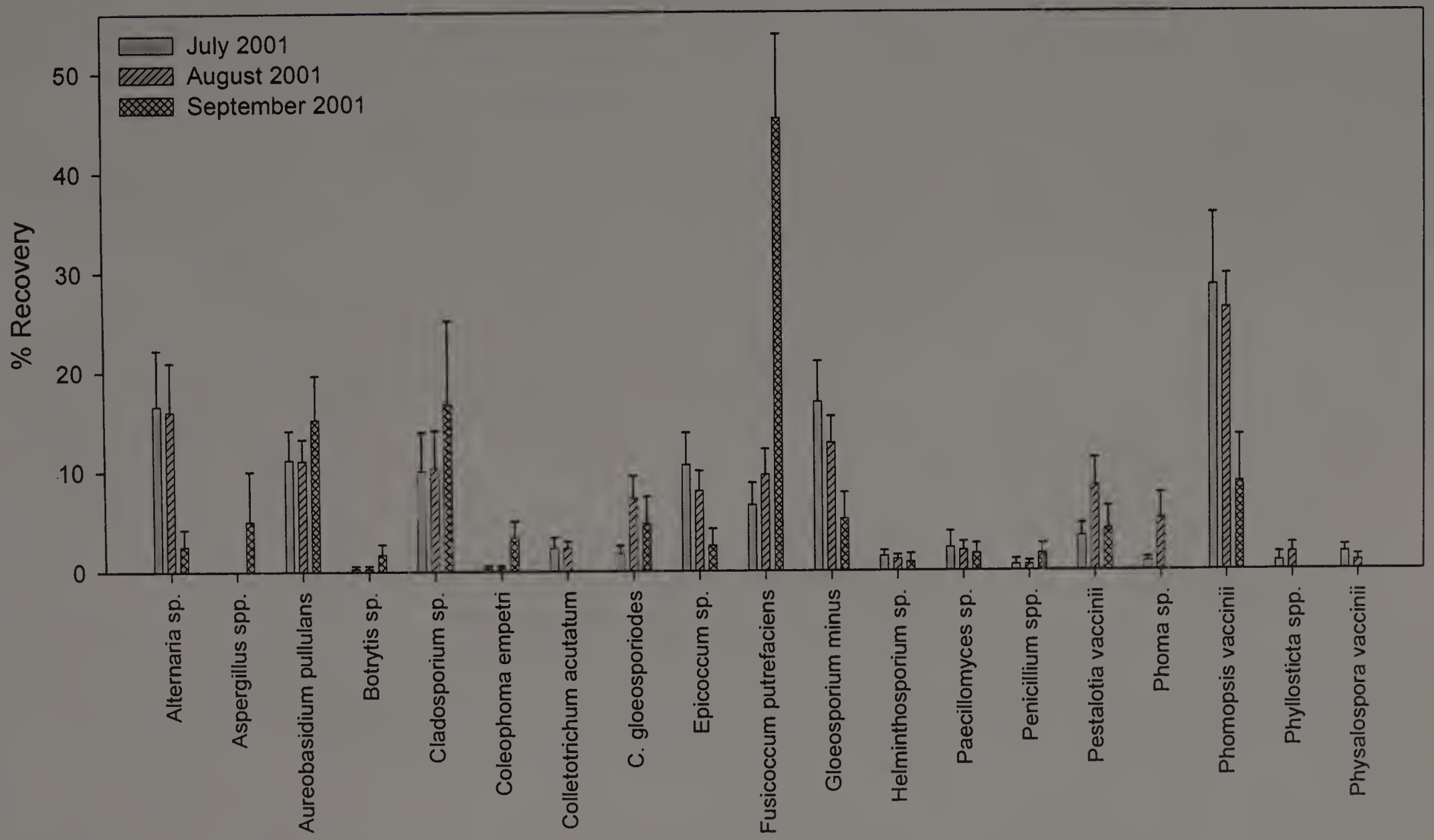


Figure A.5. Percent recovery of fungi isolated in 2001 from three collections of diseased uprights (N=7). Vertical bars represent standard error of the means.

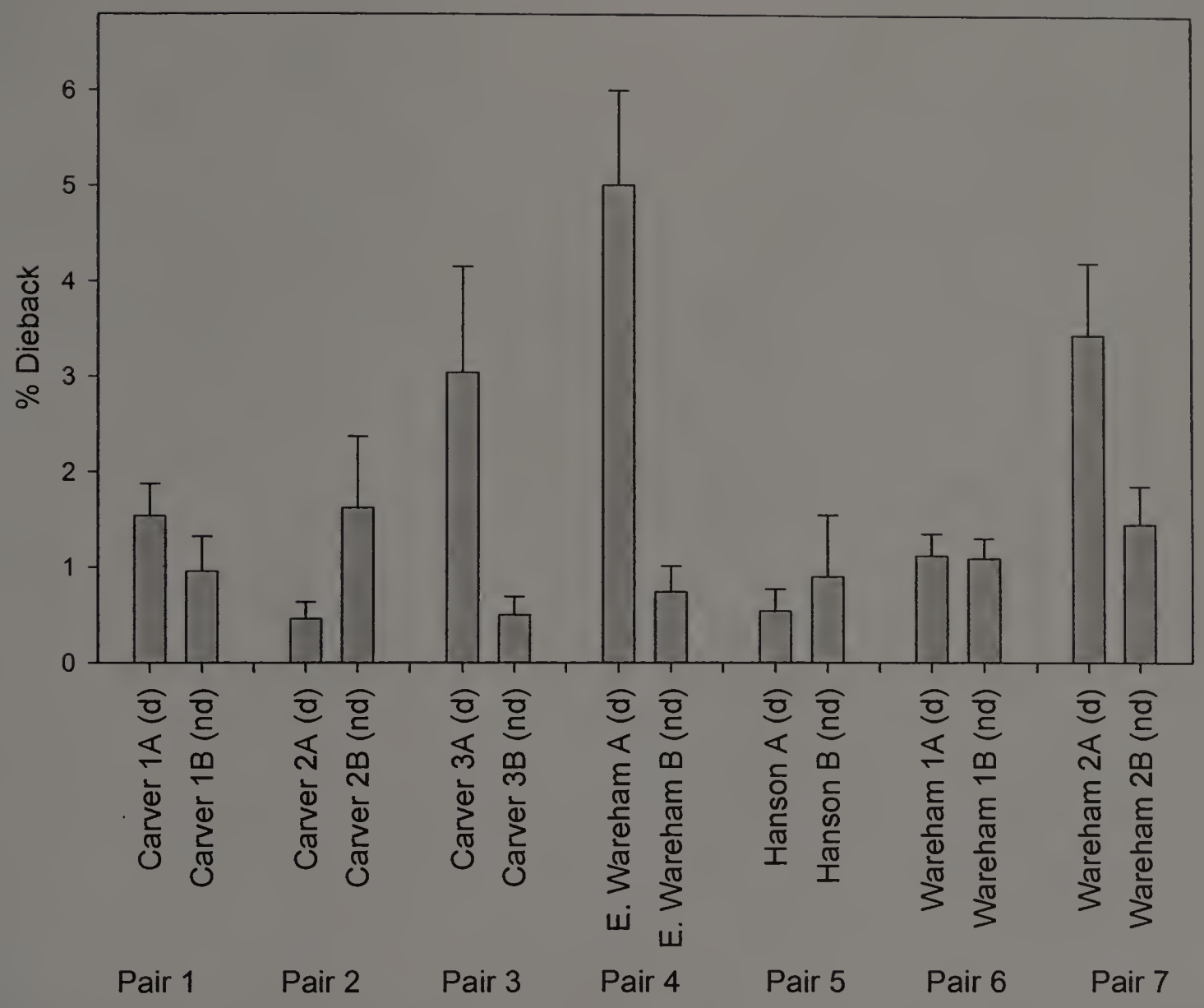


Figure A.6. Incidence of upright dieback in 2001 on seven pairs of beds (N=10). Each pair of beds consisted of one bed with a history of dieback (d) and one bed without a history of upright dieback (nd). Vertical bars represent standard error of the means. Disease incidence was significantly different between the beds of Pair 3 ($p = 0.0476$), Pair 4 ($p = 0.0006$), and Pair 7 ($p = 0.0329$).

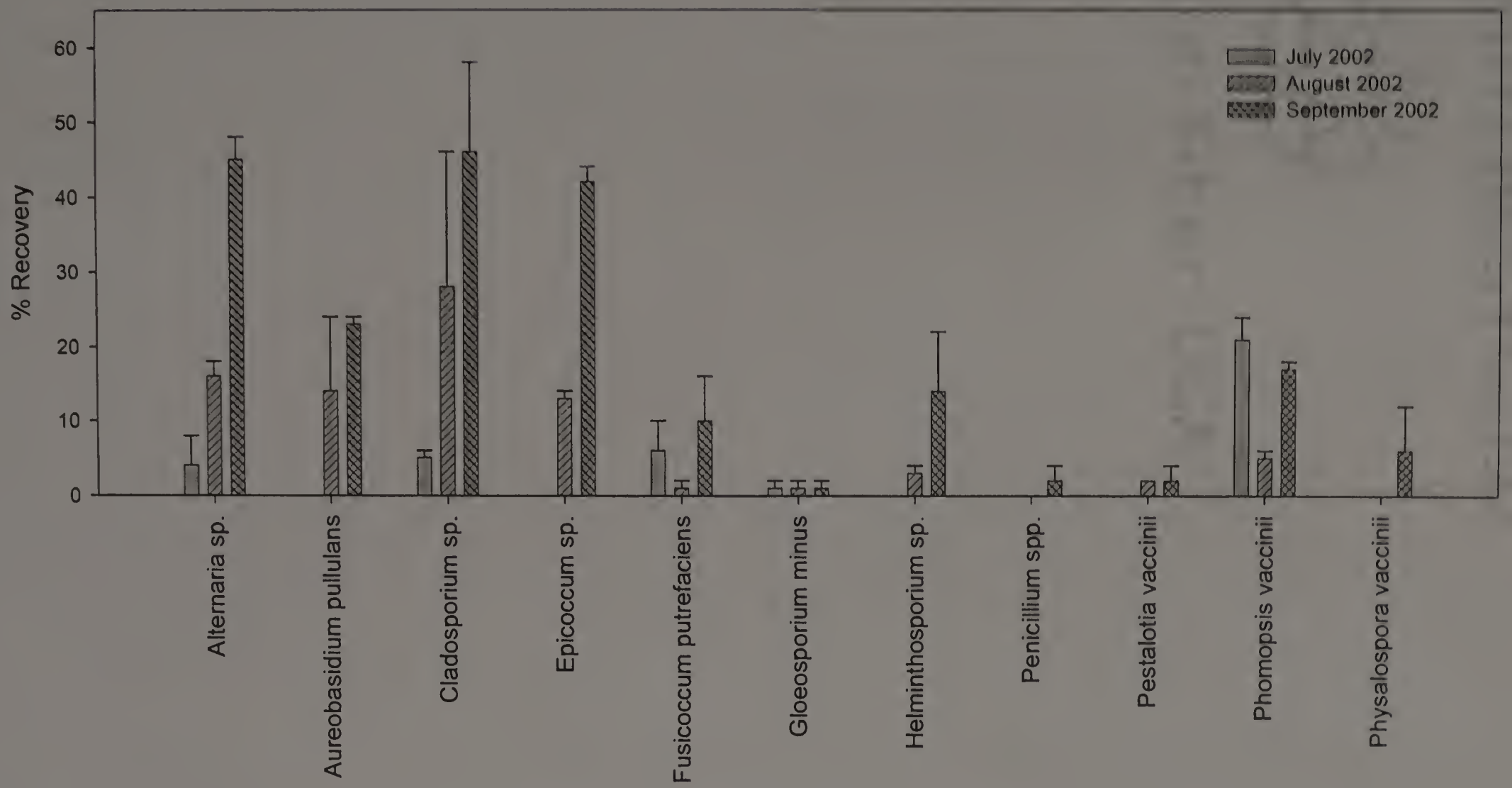


Figure A.7. Percent recovery of fungi from three collections in 2002 from symptomless current-year growth sampled from beds with a history of upright dieback (N=3). Vertical bars represent standard error of the means.

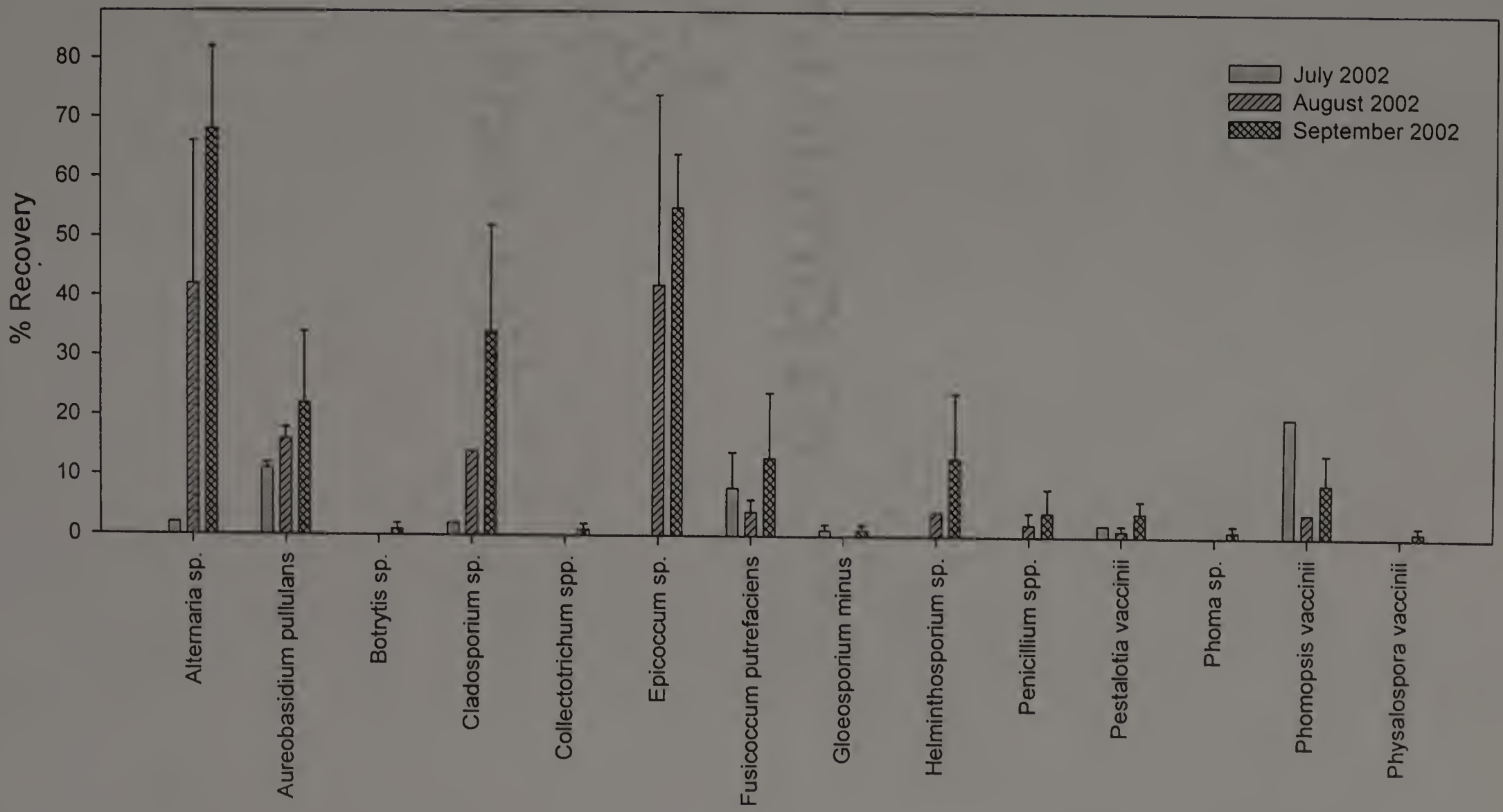


Figure A.8. Percent recovery of fungi from three collections in 2002 from symptomless current-year growth sampled from beds without a history of upright dieback (N=3). Vertical bars represent standard error of the means.

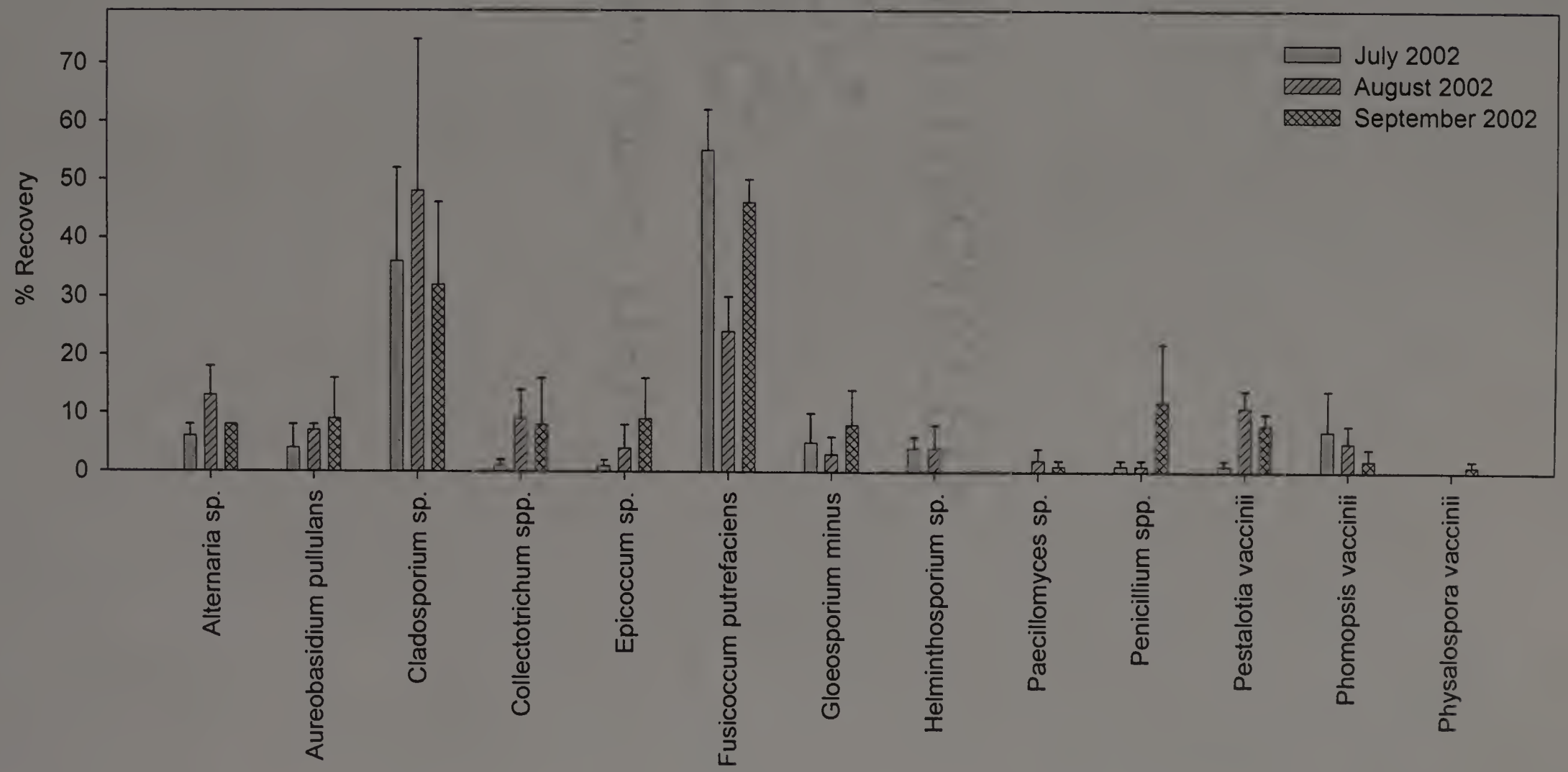


Figure A.9. Percent recovery of fungi from three collections in 2002 from symptomless one-year-old growth sampled from beds with a history of upright dieback (N=3). Vertical bars represent standard error of the means.

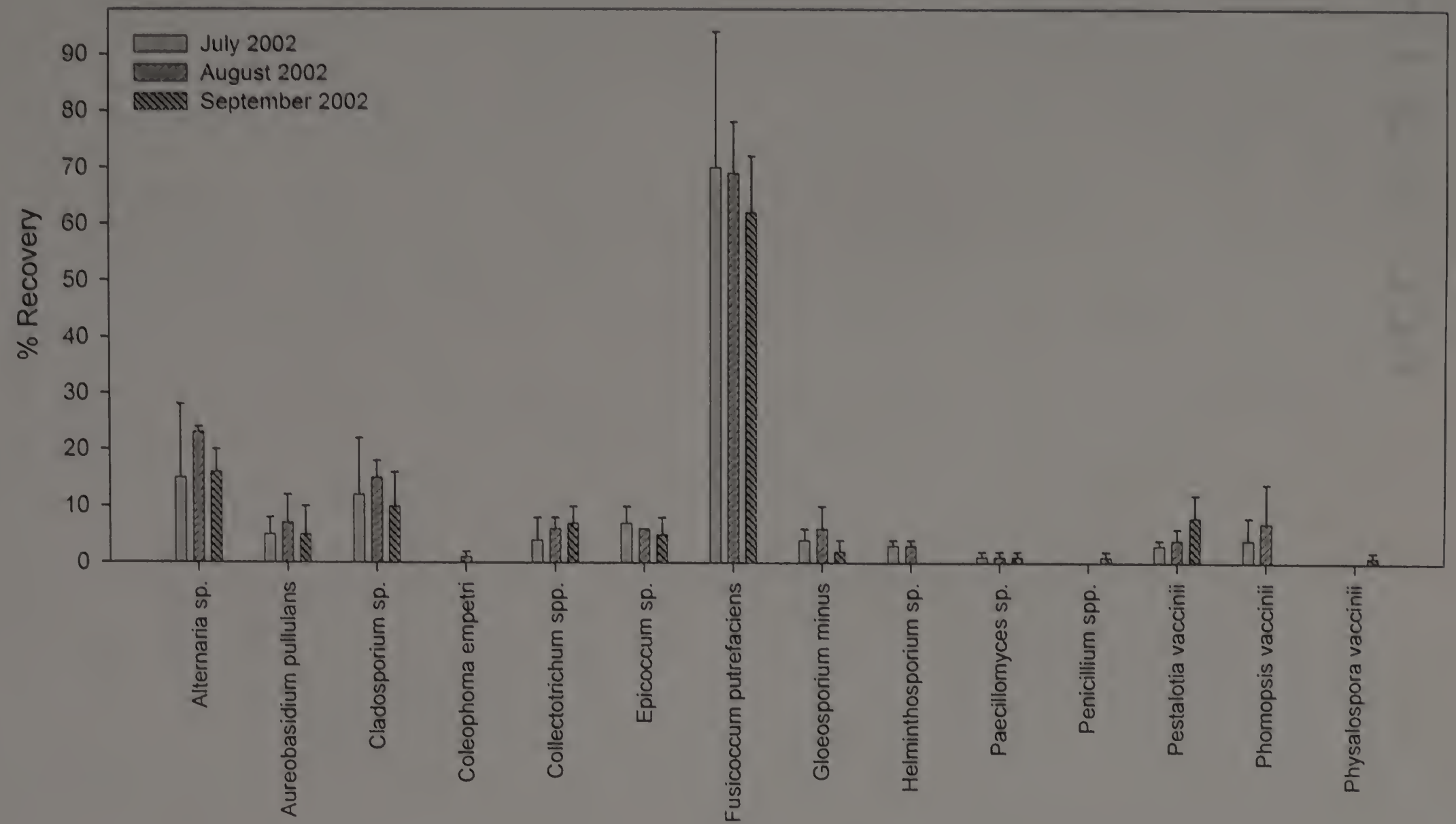


Figure A.10. Percent recovery of fungi from three collections in 2002 from symptomless one-year-old growth sampled from beds without a history of upright dieback (N=3). Vertical bars represent standard error of the means.

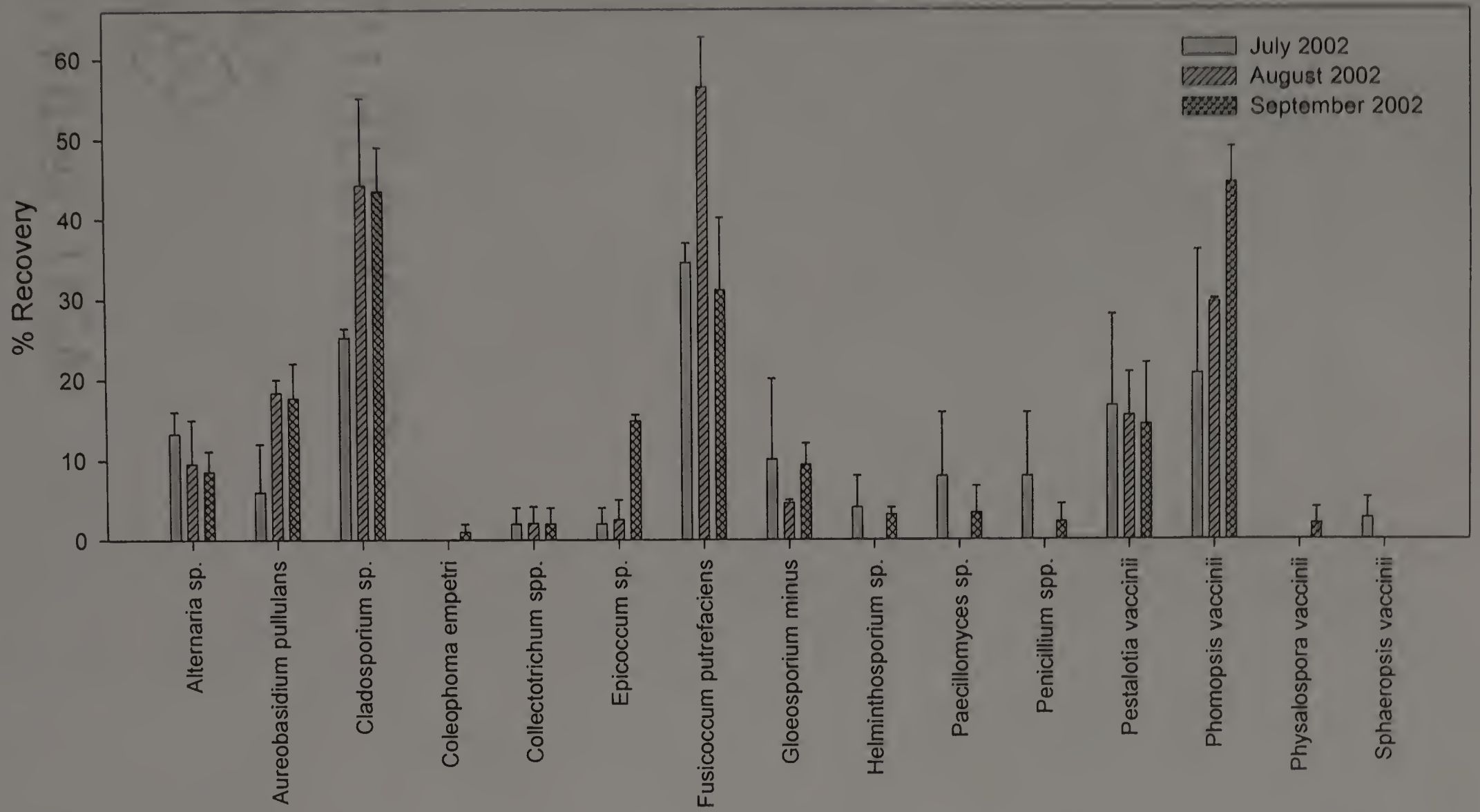


Figure A.11. Percent recovery of fungi isolated in 2002 from three collections of diseased uprights (N=3). Vertical bars represent standard error of the means.

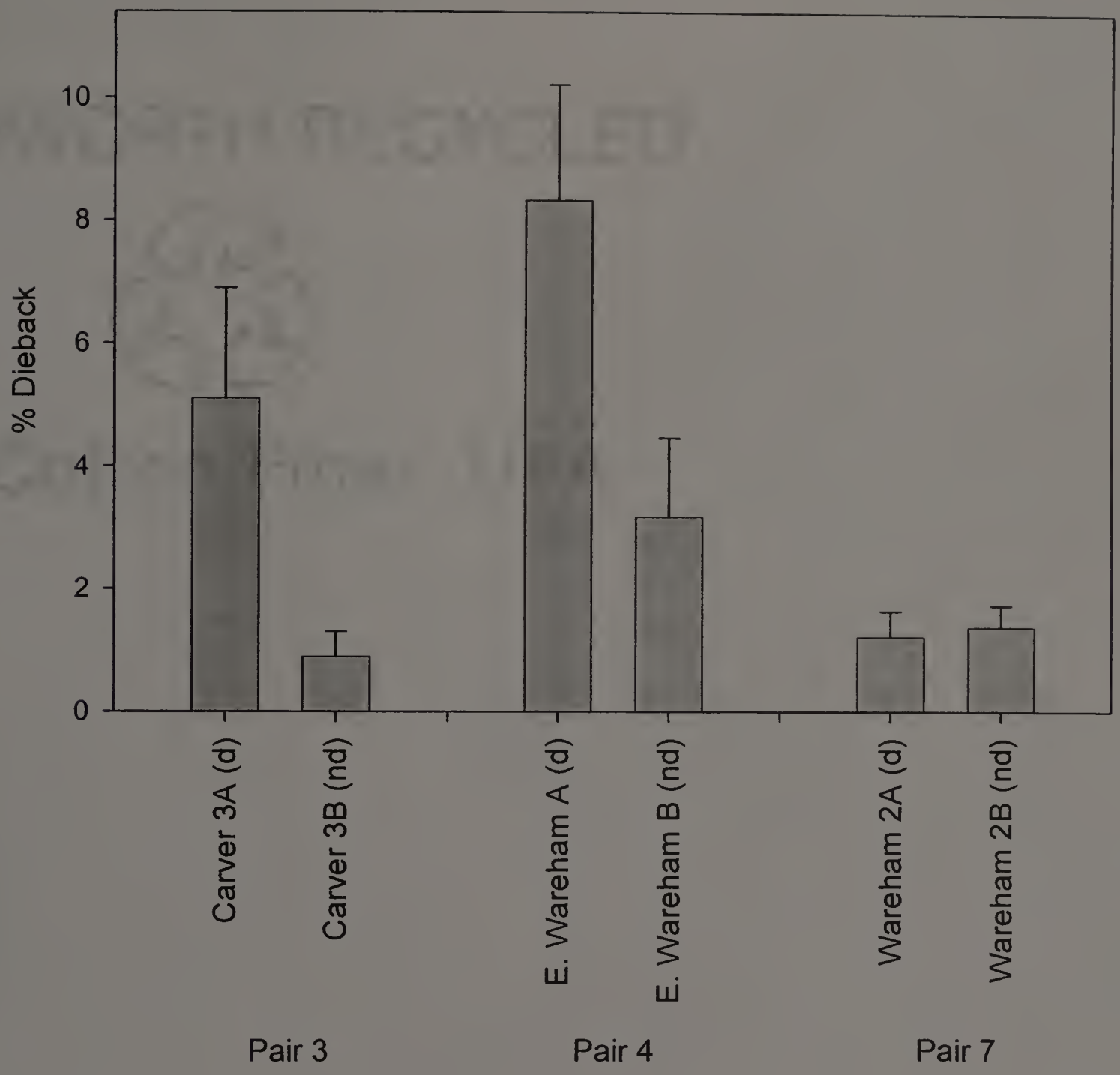


Figure A.12. Incidence of upright dieback in 2002 on three pairs of beds (N=10). Each pair of beds consisted of one bed with a history of dieback (d) and one bed without a history of upright dieback (nd). Vertical bars represent standard error of the means. Disease incidence was significantly different between the beds of Pair 3 ($p = 0.0328$) and Pair 4 ($p = 0.0360$).

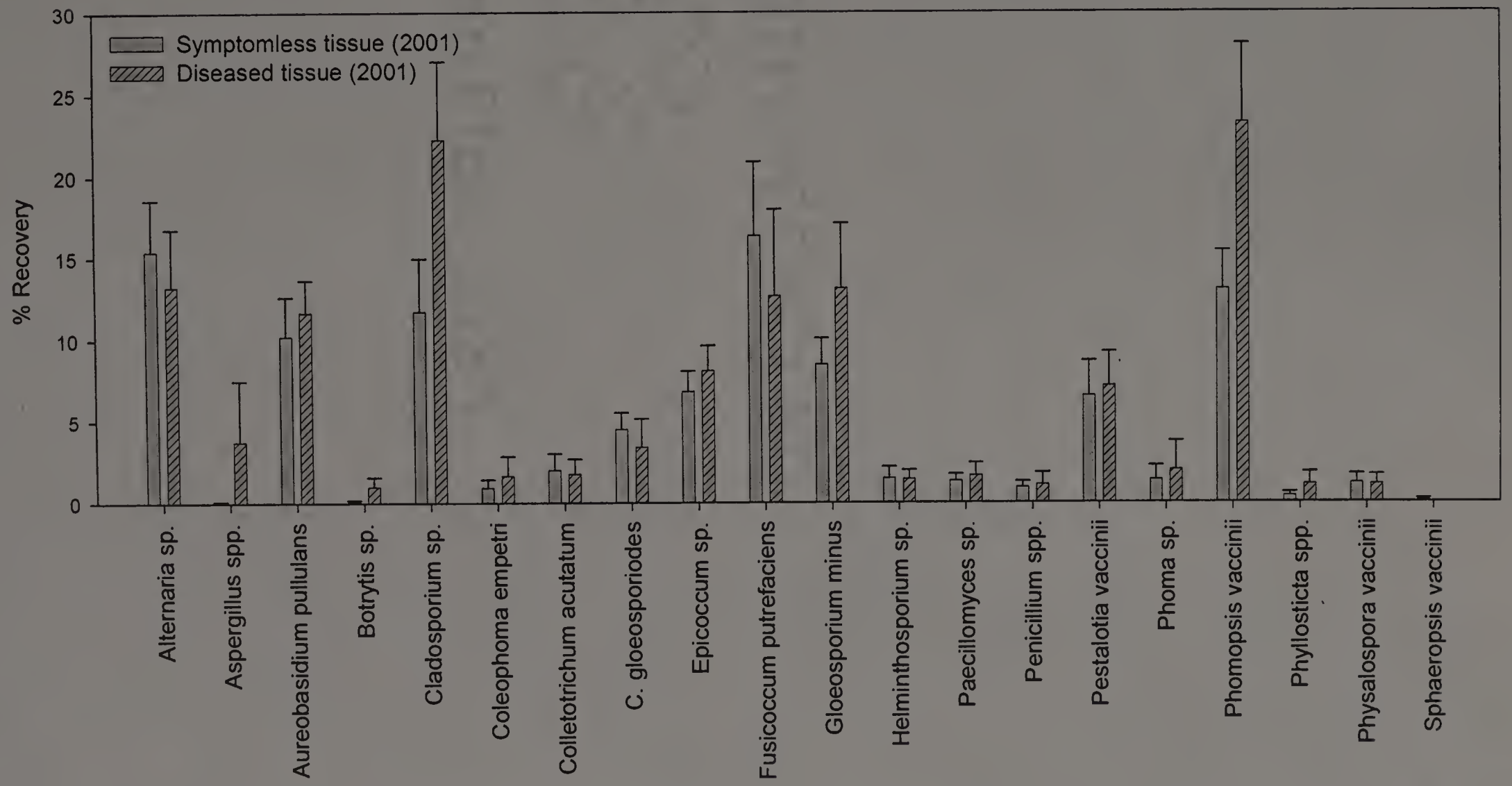


Figure A.13. Percent recovery of fungi from symptomless tissue samples (N=36) and diseased tissue samples (N=9) in 2001. Vertical bars represent standard error of the means.

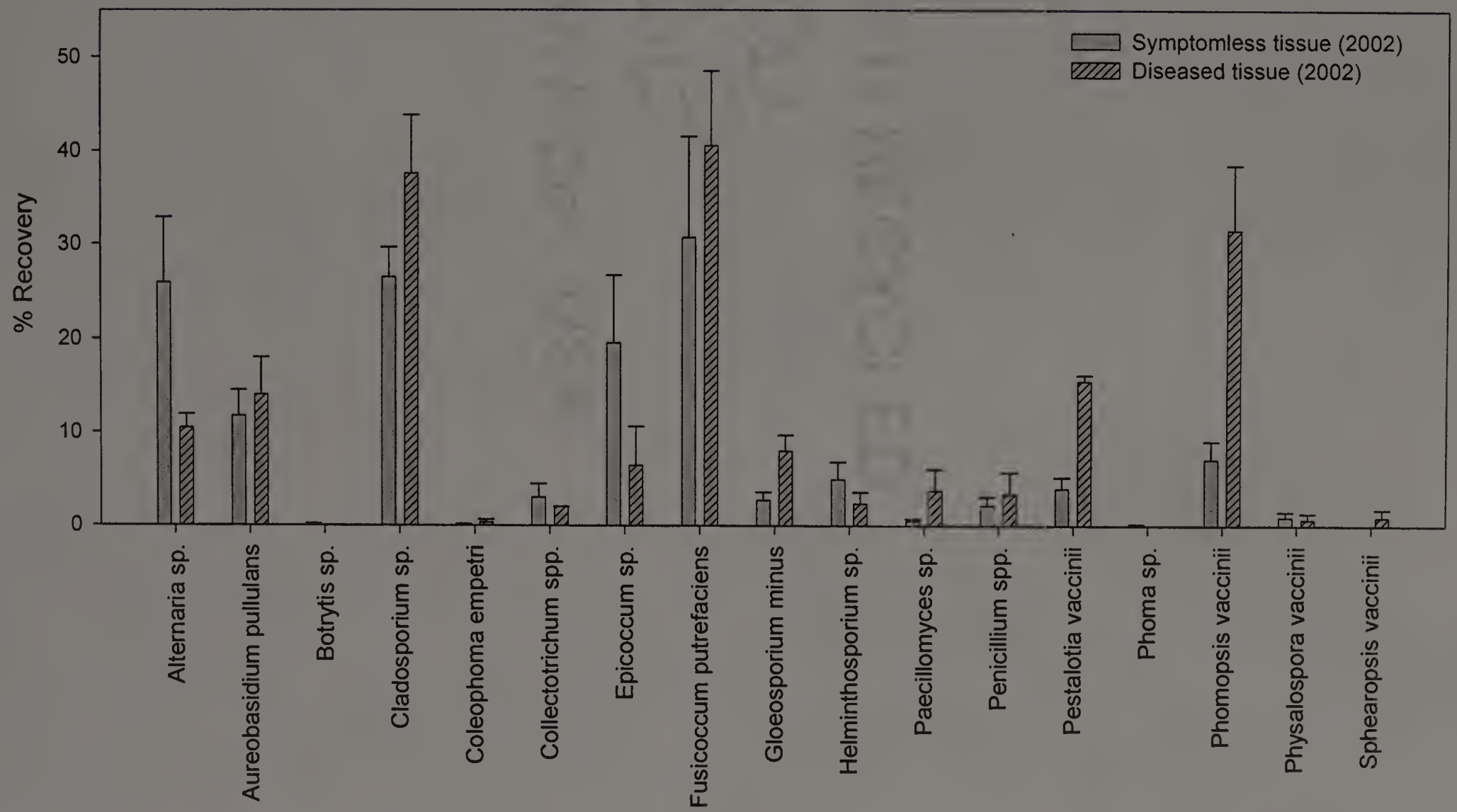


Figure A.14. Percent recovery of fungi from symptomless tissue samples (N=24) and diseased tissue samples (N=6) in 2002. Vertical bars represent standard error of the means.

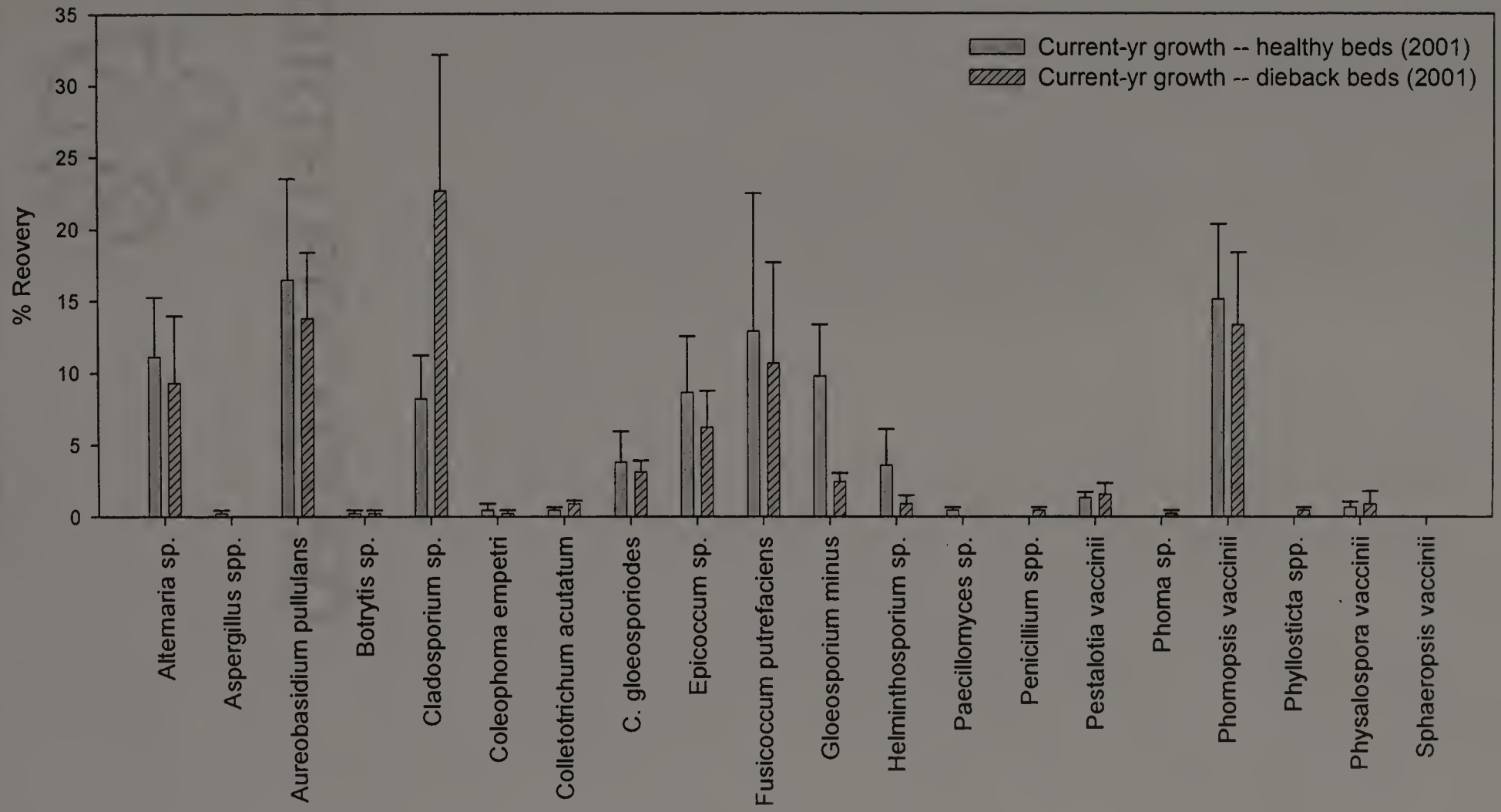


Figure A.15. Percent recovery of fungi from symptomless current-year growth sampled from beds with a history of upright dieback and beds without a history of upright dieback in 2001 (N=9). Vertical bars represent standard error of the means.

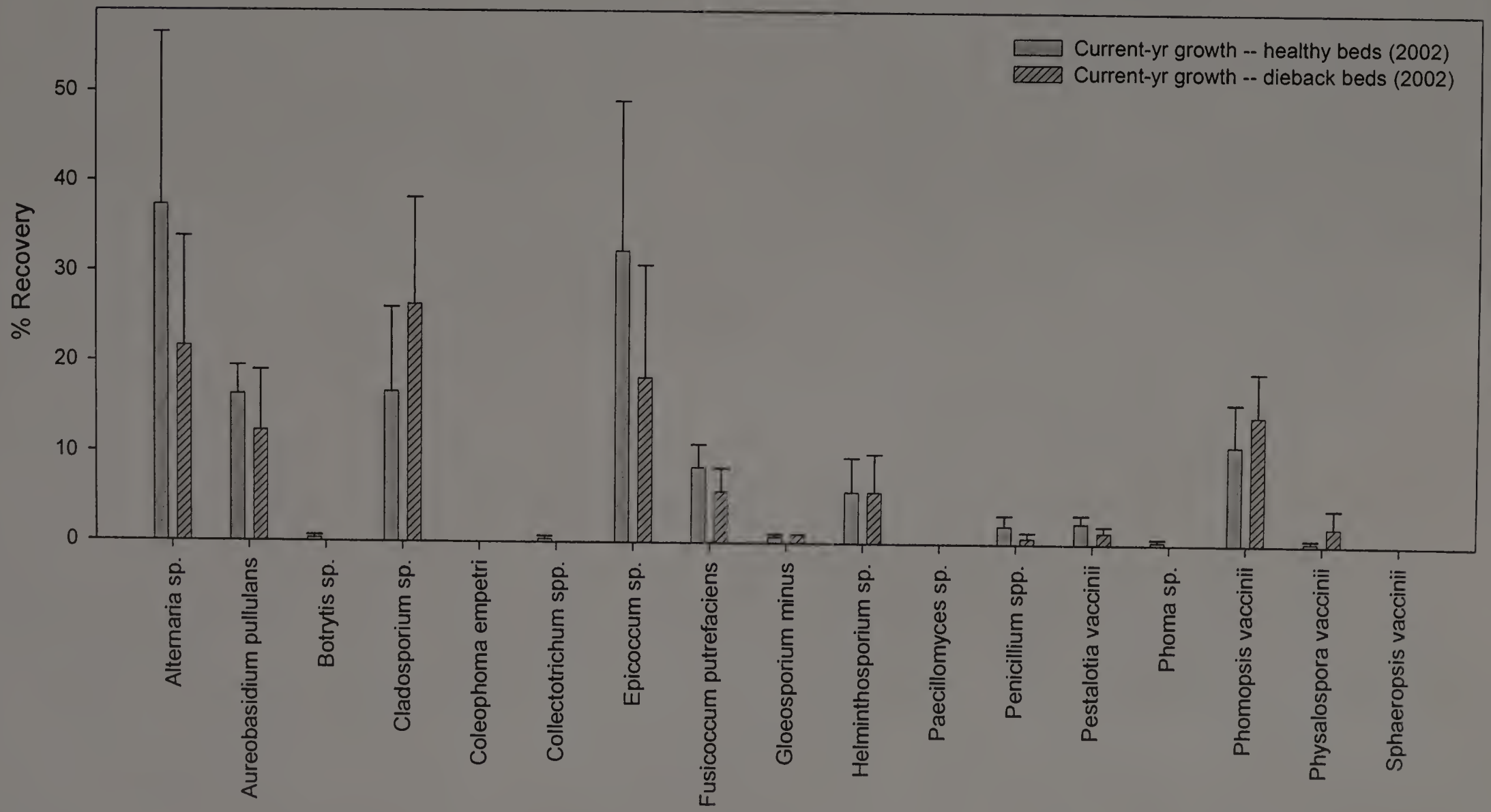


Figure A.16. Percent recovery of fungi from symptomless current-year growth sampled from beds with a history of upright dieback and beds without a history of upright dieback in 2002 (N=6). Vertical bars represent standard error of the means.

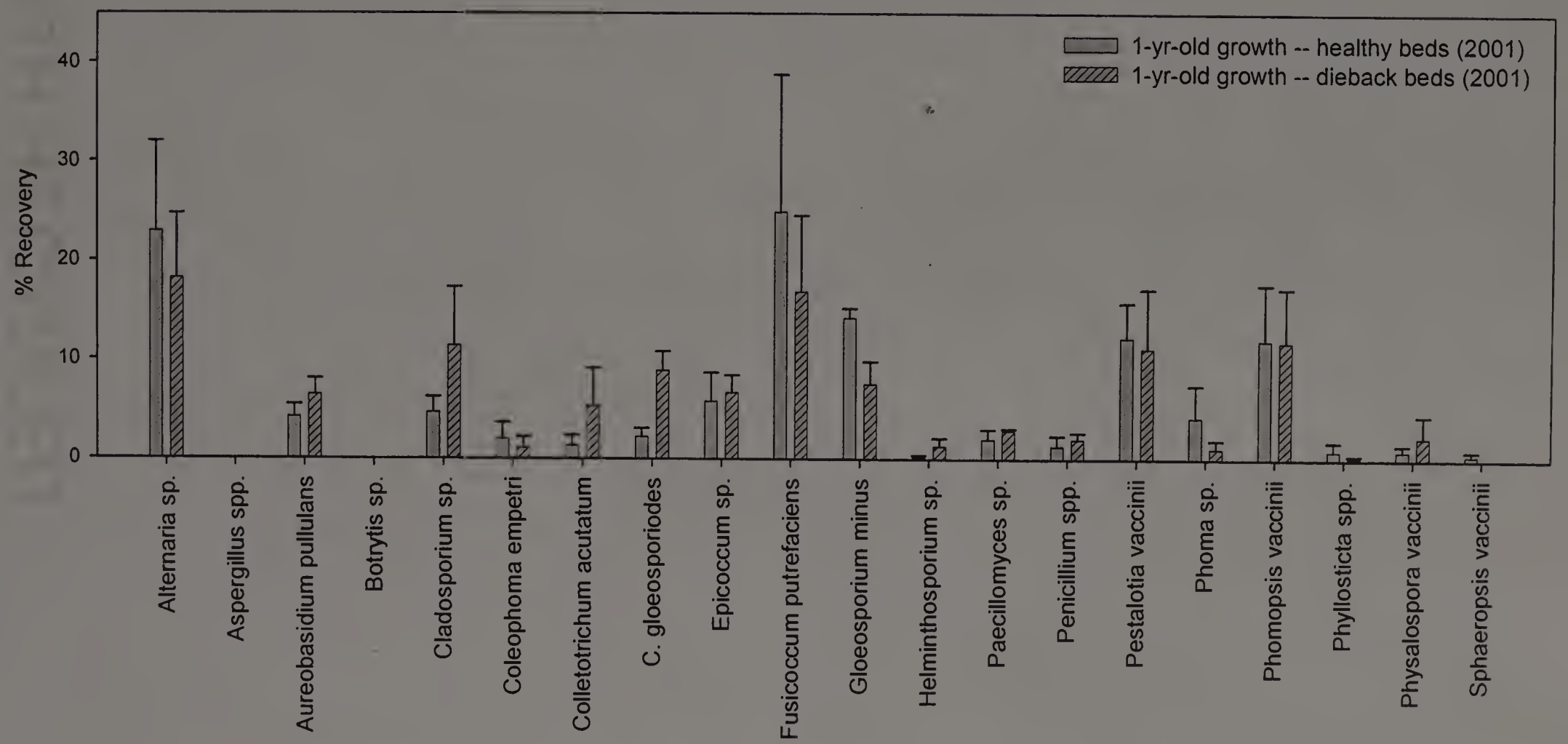


Figure A.17. Percent recovery of fungi from symptomless one-year-old growth sampled from beds with a history of upright dieback and beds without a history of upright dieback in 2001 (N=9). Vertical bars represent standard error of the means.

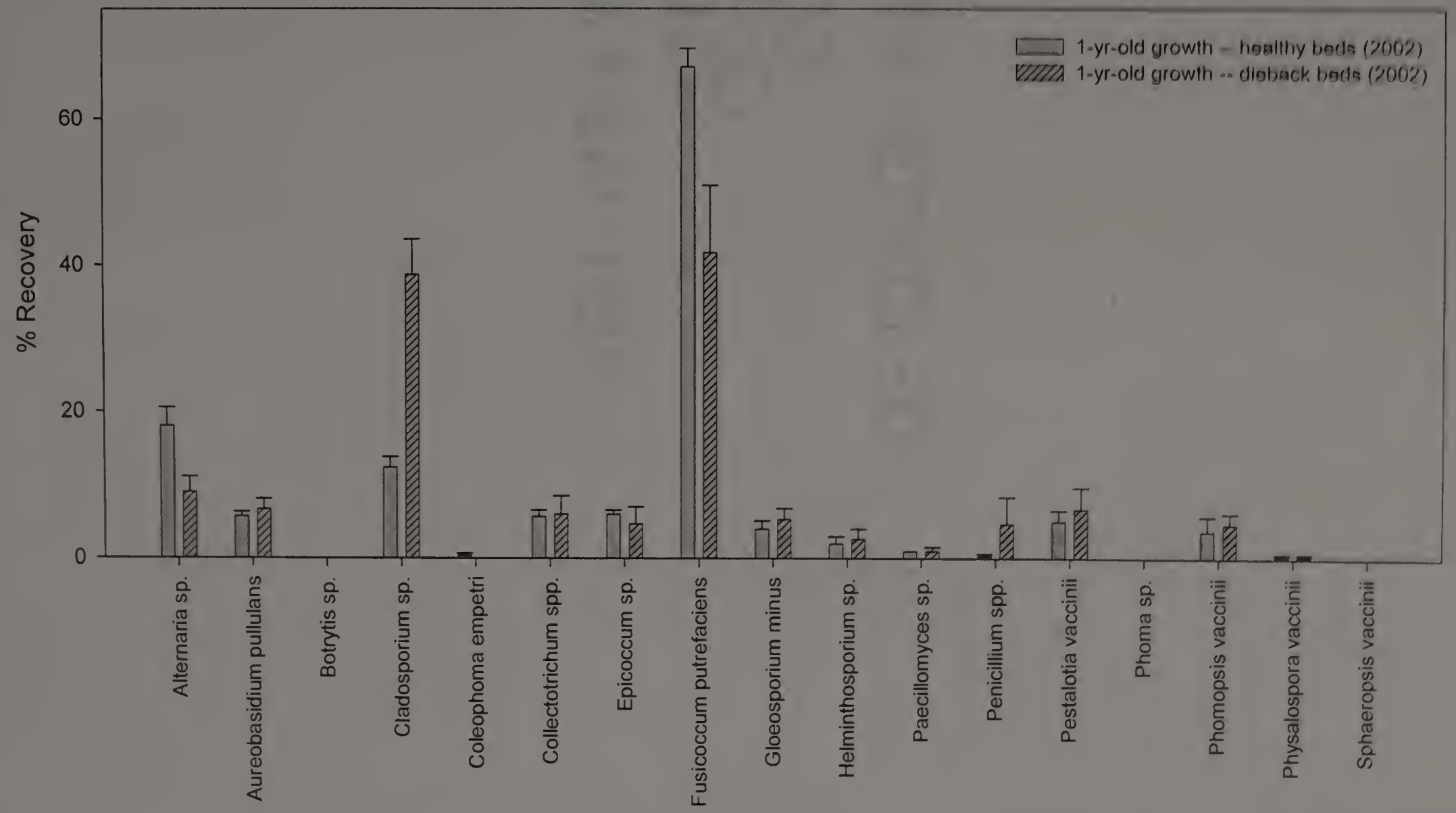


Figure A.18. Percent recovery of fungi from symptomless one-year-old growth sampled from beds with a history of upright dieback and beds without a history of upright dieback in 2002 (N=6). Vertical bars represent standard error of the means.

Table A.2. Summary of p-values from ANOVA for percent recovery from 5 types of tissue samples and 3 collection times, including interaction, for 2001 survey. NS = p>0.10.

	Tissue Sample	Collection	Tissue Sample*Collection
Aureobasidium pullulans	0.0691	0.0440	0.0549
Cladosporium sp.	NS	NS	0.0005
Colletotrichum acutatum	NS	0.0591	0.0731
Colletotrichum gloeosporiodes	NS	NS	NS
Fusicoccum putrefaciens	NS	0.0017	NS
Gloeosporium minus	NS	NS	0.0085
Pestalotia vaccinii	NS	0.0931	NS
Phomopsis vaccinii	0.0455	0.0114	NS

Table A.3. Summary of p-values from ANOVA for percent recovery from 5 types of tissue samples and 3 collection times, including interaction, for 2002 survey. NS = p>0.10.

	Tissue Sample	Collection	Tissue Sample*Collection
Aureobasidium pullulans	NS	NS	0.0337
Cladosporium sp.	NS	0.0195	0.0619
Colletotrichum spp.	NS	NS	NS
Fusicoccum putrefaciens	0.0259	NS	NS
Gloeosporium minus	NS	NS	NS
Pestalotia vaccinii	0.0464	0.0891	NS
Phomopsis vaccinii	0.1069	NS	0.0804

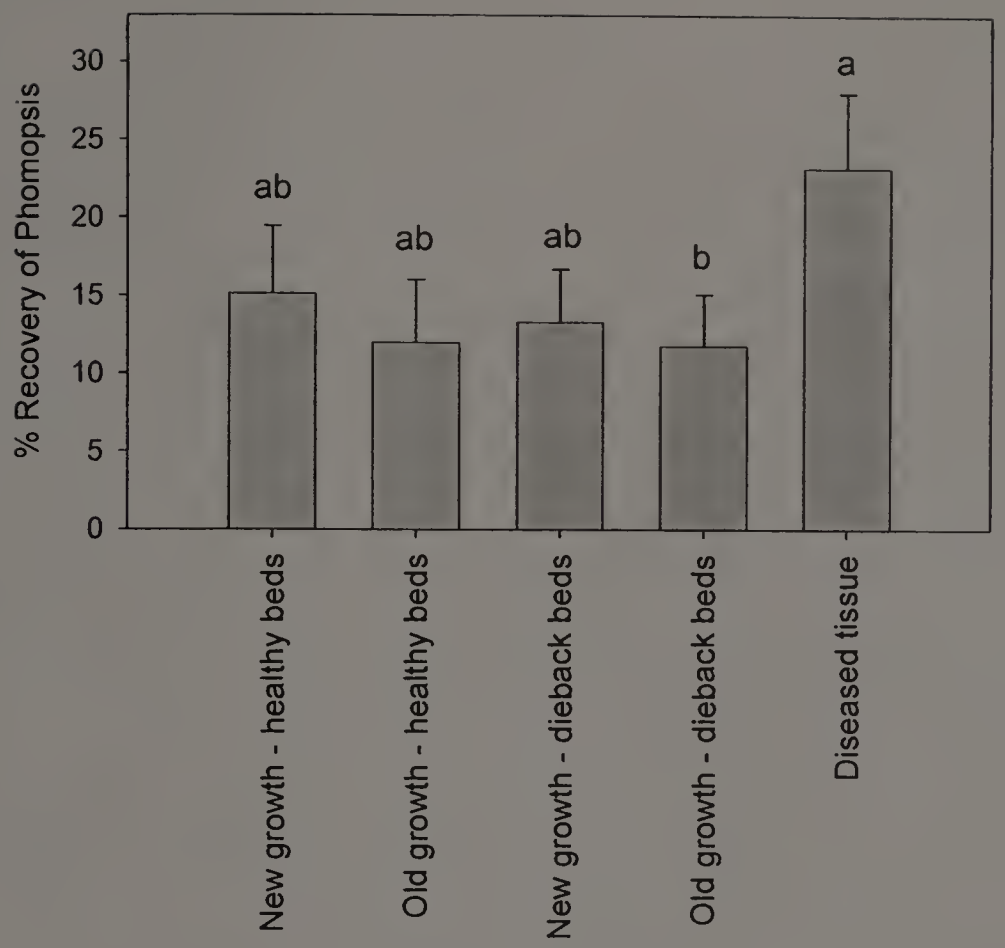


Figure A.19. Percent recovery of *P. vaccinii* from different tissue samples in 2001 (N=9). Vertical bars represent standard error of the means. Means with similar letters are not significantly different according to Kramer-adjusted Tukey's HSD (p = 0.05).

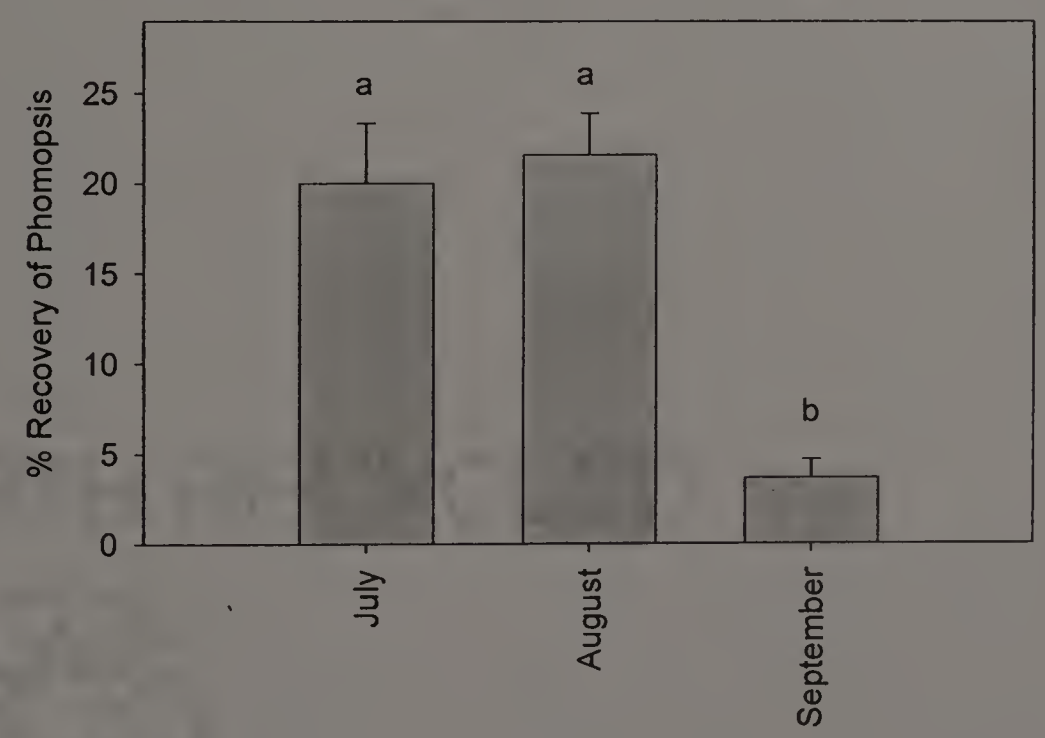


Figure A.20. Percent recovery of *P. vaccinii* from different collection times in 2001 (N=15). Vertical bars represent standard error of the means. Means with similar letters are not significantly different according to Kramer-adjusted Tukey's HSD (p = 0.05).

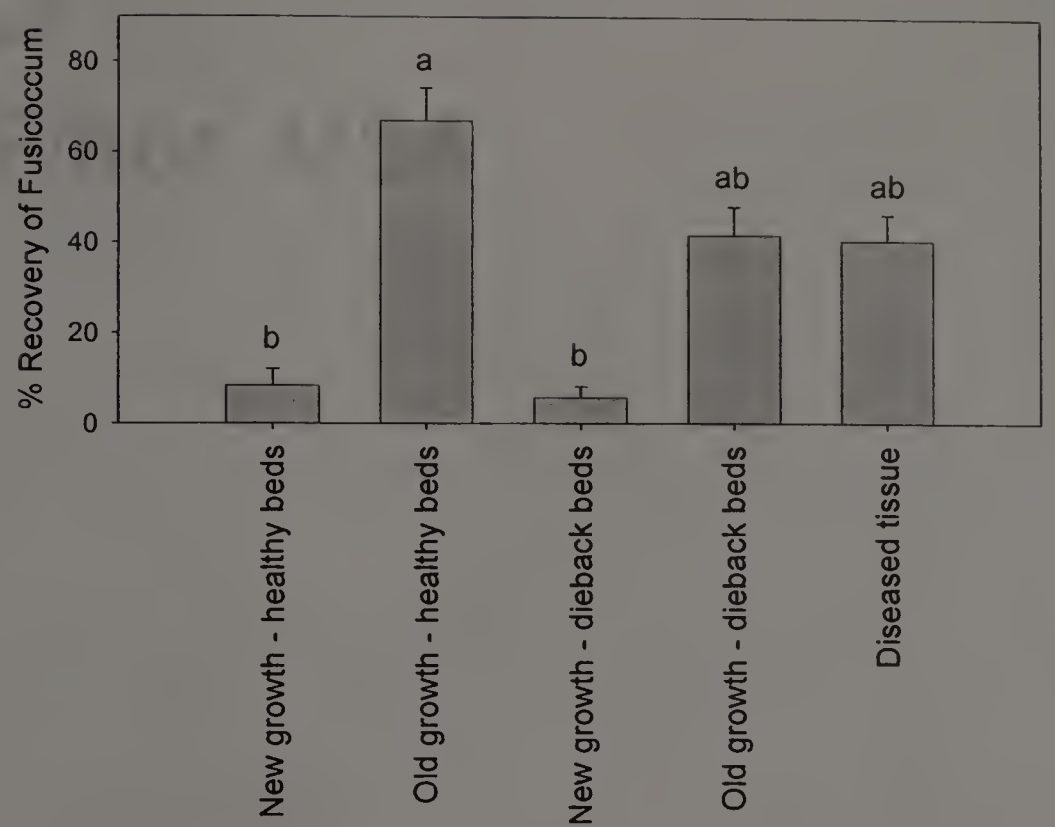


Figure A.21. Percent recovery of *F. putrefaciens* from different tissue samples in 2002 (N=6). Vertical bars represent standard error of the means. Means with similar letters are not significantly different according to Kramer-adjusted Tukey's HSD (p = 0.05).

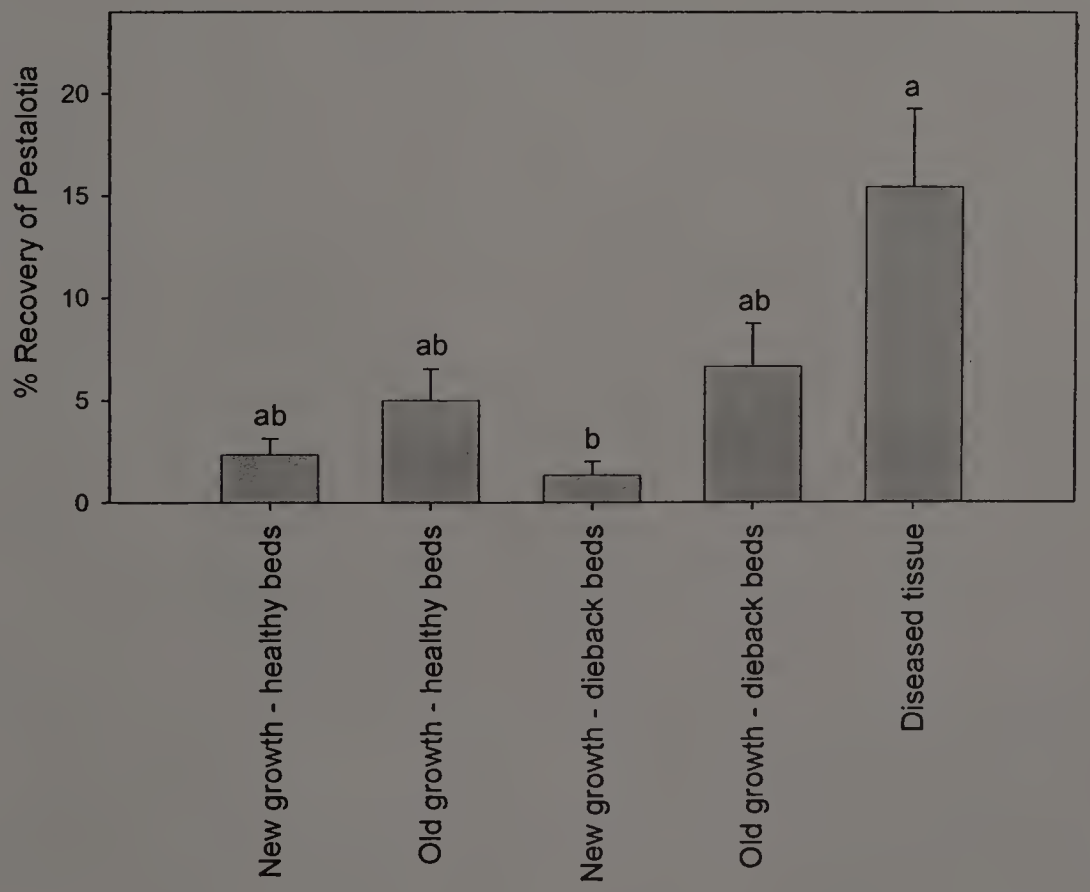


Figure A.22. Percent recovery of *Pestalotia* sp. from different tissue samples in 2002 (N=6). Vertical bars represent standard error of the means. Means with similar letters are not significantly different according to Kramer-adjusted Tukey's HSD (p = 0.05).

REFERENCES

Abdollahi, H., Rugini, E., Ruzzi, M., and Muleo, R. 2004. *In vitro* system for studying the interaction between *Erwinia amylovora* and genotypes of pear. Plant Cell, Tissue Organ Cult. 79:203-212.

Agrios, G.N. 1988. Plant Pathology, third edition. Academic Press, San Diego, CA.

Baker, J.B., Hancock, J.F., and Ramsdell, D.C. 1995. Screening highbush blueberry cultivars for resistance to Phomopsis canker. HortScience 30:586-588.

Caruso, F.L. University of Massachusetts Cranberry Station, East Wareham, MA. Unpublished data.

Caruso, F.L. 2005. University of Massachusetts Cranberry Station, East Wareham, MA. Personal communication.

Caruso, F.L. 2005. Disease Management. Pages 1-5 in: M.M. Sylvia and D. Cannon, eds. Cranberry Chart Book – Management Guide for Massachusetts. UMass Cranberry Sta. Ext. Publ., East Wareham, MA.

Caruso, F.L. and Ramsdell, D.C., eds. 1995. Compendium of Blueberry and Cranberry Diseases. American Phytopathological Society, St. Paul, MN.

Castlebury, L.A. 2005. USDA ARS Systematic Botany and Mycology Laboratory, Beltsville, MD. Personal communication.

Catlin, N.J. and Caruso, F.L. University of Massachusetts Cranberry Station, East Wareham, MA. Unpublished data.

Catlin, N.J. and F.L. Caruso. 2001. Further insight into the causal agent(s) of upright dieback of cranberry. Phytopathology 91:S14.

Chao, C.P. and Glawe, D.A. 1985. Studies on the taxonomy of *Diaporthe vaccinii*. Mycotaxon 23:371-381.

Dana, M.N., Boone, D.M., Friend, R.J. and Koval, C.M. 1967. The controversial case of cranberry vine injury. Cranberries 31:16, 32.

Daykin, M.E. and Milholland, R.D.. 1990. Histopathology of blueberry twig blight caused by *Phomopsis vaccinii*. Phytopathology 80:736-740.

DeMoranville, C.J. 2001. Reducing Management Costs in Cranberry Production. UMass Cranberry Sta. Ext. Publ., East Wareham, MA.

DeMoranville, C.J. and Sandler, H.A. 2000. Best management practices guide for cranberry production in Massachusetts. UMass Cranberry Sta. Ext. Publ., East Wareham, MA.

DeMoranville, C.J., Averill, A.L., Caruso, F.L., and Sandler, H.A. 2005. Later water and cranberry management. Pages 1-5 in: M.M. Sylvia and D. Cannon, eds. Cranberry Chart Book – Management Guide for Massachusetts. UMass Cranberry Sta. Ext. Publ., East Wareham, MA.

DeMoranville, C.J., DeMoranville, I.E., and Caruso, F.L. 1997. Influence of weather on cranberry crop prediction and quality, Pages in: H.A. Sandler, ed. Cranberry Production – A Guide for Massachusetts. UMass Cranberry Sta. Ext. Publ., East Wareham, MA.

Eck, P. 1990. The American Cranberry. Rutgers Univ. Press, New Brunswick, NJ.

Espinosa-Garcia, F.J. and Langenheim, J.H. 1990. The endophytic fungal community in leaves of a coastal redwood population – diversity and spatial patterns. New Phytol. 116:89-97.

Farr, D.F., Castlebury, L.A., and Rossman, A.Y. 2002. Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of *Phomopsis* from blueberry and cranberry in the eastern United States. Mycologia 94:494-504.

Friend, R.J. and Boone, D.M. 1968. *Diaporthe vaccinii* associated with dieback of cranberry in Wisconsin. Plant Dis. Rep. 52:341-344.

Hammerschlag, F.A. 1988. Screening peaches in vitro for resistance to *Xanthomonas campestris* pv. *pruni*. J. Amer. Soc. Hort. Sci. 113:164-166.

Levesque, C.A., Harlton, C.E. and de Jong, S. 1999. The use of DNA arrays for multiplex detection of fungi in plant tissue and on rotorod spore samplers. Phytopathology 89:S45.

Lloyd, G. and McCown, B. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Intl. Plant Prop. Soc. 30:421-427.

Mahr, S.E.R. and Moffitt, L.J. 1994. Biologic and economic assessment of pesticide usage in the cranberry industry. NAPIAP Report No. 2-CA-94.

Milholland, R.D. 1982. Blueberry twig blight caused by *Phomopsis vaccinii*. Plant Dis. 66:1034-1036.

Milholland, R.D. and Daykin, M.E. 1983. Blueberry fruit rot caused by *Phomopsis vaccinii*. Plant Dis. 67:325-326.

Mostert, L., Crous. P.W., and Petrini, O. 2000. Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. Sydowia 52:46-58.

Muntanola-Cvetkovic, M., Vukojevic, J. and Mihaljcevic, M. 1989. Pathohistology of sunflower stems attacked by *Diaporthe helianthi*. Can. J. Bot. 67:1119-1125.

Muntanola-Cvetkovic, M., Vukojevic, J. and Mihaljcevic, M. 1991. The systemic nature of the sunflower disease caused by *Diaporthe helianthi*. Can. J. Bot. 69:1552-1556.

New England Agricultural Statistics Service. 2005. Concord, NH.

Oudemans, P.V., Caruso, F.L and Stretch, A.W. 1998. Cranberry fruit rot in the Northeast: a complex disease. Plant Dis. 82:1176-1184.

Parker, P.E. and Ramsdell, D.C. 1977. Epidemiology and chemical control of Phomopsis canker of highbush blueberry. Phytopathology 67:1481-1484.

Petrini, O. 1984. Endophytic fungi in British Ericaceae: a preliminary study. Trans. Br. Mycol. Soc. 83:510-512.

Petrini, O. 1991. Fungal endophytes of tree leaves. Pages 179-197 in: Microbial Ecology of Leaves. J.H. Andrews and S.S. Hirano, eds. Springer, New York.

Ragazzi, A., Moricca, S., Capretti, P., and Dellavalle, I. 1999. Endophytic presence of *Discula quercina* on declining *Quercus cerris*. Phytopath. Z. 147:437-440.

Rawnsley, B., Wicks, T.J., Scott, E.S., and Stummer, B.E. 2004. *Diaporthe perjuncta* does not cause Phomopsis cane and leaf spot disease of grapevine in Australia. Plant Dis. 88:1005-1010.

Rehner, S.A. and Uecker, F. A. 1994. Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. Can. J. Bot. 72:1666-1674.

Sahashi, N., Kubono, T., Miyasawa, Y., and Ito, S. 1999. Temporal variations in isolation frequency of endophytic fungi of Japanese beech. Can. J. Bot. 77:197-202.

Sahashi, N., Miyasawa, Y., Kubono, T., and Ito, S. 2000. Colonization of beech leaves by two endophytic fungi in northern Japan. For. Pathol. 30:77-86.

Saikkonen, K., Wali, P., Helander, M., and Faeth, S.H. 2004. Evolution of endophyte-plant symbioses. Trends Plant Sci. 9:275-280.

Saikkonen, K., Faeth, S.H., Helander, M., and Sullivan, T.J. 1998. Fungal endophytes: a continuum of interactions with host plants. Ann. Rev. Ecol. Syst. 29:319-343.

Santos, C., Fragoeiro, S., and Phillips, A. 2005. Physiological response of grapevine cultivars and a rootstock to infection with *Phaeoacremonium* and *Phaeomoniella* isolates: an in vitro approach using plants and calluses. Sci. Hortic. 103:187-198.

SAS Institute. 2003. Release 9.1 (TS1M2) of the SAS System for Microsoft Windows. SAS Institute

Scheck, H.J., Canfield, M.L., Pscheidt, J.W., and Moore, L.W. 1997. Rapid evaluation of pathogenicity in *Pseudomonas syringae* pv. *syringae* with lilac tissue culture bioassay and syringomycin DNA probes. Plant Dis. 81:905-910.

Schulz, B., Guske, S., Dammann, U., and Boyle, C. 1998. Endophyte-Host Interactions. II. Defining Symbiosis of the Endophyte-Host Interaction. Symbiosis 25:213-217.

Shear, C.L., Stevens, N.E., and Bain, H.F. 1931. Fungous diseases of the cultivated cranberry. US Dept. Agric. Tech. Bull. 258:1-58.

Spanos, K.A., Pirrie, A., and Woodward, S. 1997. *In vitro* expression of resistance responses to *Seiridium* species in micropropagated shoots of *Cupressus sempervirens* and *Chamaecyparis lawsoniana*. Can. J. Bot. 75:1103-1109.

Stiles, C.M. and Oudemans, P.V. 1999. Distribution of cranberry fruit-rotting fungi in New Jersey and evidence for nonspecific host resistance. Phytopathology 89:218-225.

Toti, L., Viret, O., Horat, G., and Petrini, O. 1993. Detection of the endophyte *Discula umbrinella* in buds and twigs of *Fagus sylvatica*. Eur. J. For. Path. 23:147-152.

Uecker, F.A. 1988. A world list of *Phomopsis* names with notes on nomenclature, morphology, and biology. Mycol. Mem. No. 13.

Uecker, F. A., and Caruso, F.L. 1988. *Synchronoblastia crypta*, a new coelomycetous pathogen of upright stems and fruits of cranberry. Mycologia 80:344-347.

Verhoeff, K. 1974. Latent infections by fungi. Annu. Rev. Phytopathol. 12:99-110.

Weingartner, D.P. and Klos, E.J. 1975. Etiology and symptomalogy of canker and dieback diseases on highbush blueberries caused by *Godronia* (*Fusicoccum*) *cassandrae* and *Diaporthe* (*Phomopsis*) *vaccinii*. Phytopathology 65:105-110.

Wilcox, M.S. 1939. Phomopsis twig blight of blueberry. Phytopathology 29:136-142.

Wilcox, M.S. 1940. *Diaporthe vaccinii*, the ascigerous stage of *Phomopsis*, causing a twig blight of blueberry. Phytopathology 30:441-443.

Wilson, D. 1995. Endophyte – the evolution of a term, and clarification of its use and definition. Oikos 73:274-276.

Wilson, D. and Carroll, G.C. 1994. Infection studies of *Discula quercina*, and endophyte of *Quercus garryana*. Mycologia 86:635-647.

Zilkah, S., Faingersh, E., Rotbaum, A., Dvorkin, R., Eilam, T., and Anikster, Y. 2000. Tissue culture of the alternate hosts of wheat, rye and oat rusts and their response to *in vitro* inoculation with the rust pathogens. Plant Cell, Tissue Organ Cult. 59:209-215.